



British Journal of Dermatology

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British Journal of Dermatology

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P, 25, 440

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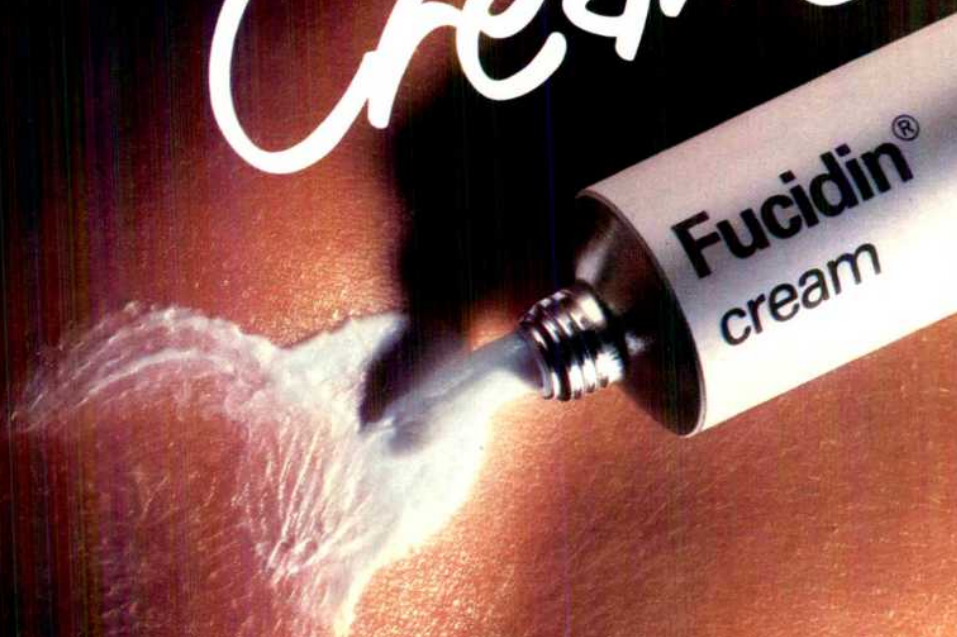
PSORIASIS

REAM PRESCRIBING INFORMATION PRESENTATION: Pale yellow aqueous cream containing: Dithrocream 0.1%: 0.1% Dithranol BP. Dithrocream 0.25%: 0.25% Dithranol BP. Dithrocream Forte: 0.5% BP. Dithrocream HP: 1.0% Dithranol BP. Dithrocream 2.0%: 2.0% Dithranol BP. INDICATIONS: For the topical treatment of sub-acute and chronic psoriasis including psoriasis of the scalp. Dithrocream 0.1%, Dithrocream HP (1.0%) and Dithrocream 2.0% should only be used once daily for those patients who have failed to respond to lower strengths of dithranol. DIRECTIONS FOR USE: Dithrocream applied at any convenient time and then removed by washing off, usually no more than one hour after application. Alternatively it may be applied at night before retiring and washed off in the morning. CONTRA-INDICATIONS: Acute or pustular psoriasis, dithranol sensitivity. PRECAUTIONS: Keep away from eyes and mucous membranes. Apply to psoriatic area cause staining. PACKAGE QUANTITY AND BASIC NHS PRICES: All strengths of Dithrocream are supplied in collapsible tubes containing 50g. Dithrocream 0.1% £3.36; 0.25% £3.62; Forte 0.5% £4.17 £4.81; 2.0% £6.25. PRODUCT LICENCE NUMBERS: Dithrocream 0.1%: 0173/0029. Dithrocream 0.25% 0173/0028. Dithrocream Forte (0.5%): 0173/0027. Dithrocream HP (1.0%): 0173/0039. Dithrocream 2.0% 0173/0045. Full prescribing information is available from: Dermal Laboratories Limited, Gosmore, Hitchin, Hertfordshire SG4 7QR. Dithrocream is a registered trade mark.

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Scotia Pharmaceuticals is currently involved with original research into a number of areas including skin diseases such as atopic eczema and seborrhoeic dermatitis; complications of diabetes; rheumatoid arthritis; menstrual disorders and certain cancers.



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Studies performed by Scotia Pharmaceuticals and others have demonstrated that EPOGAM corrects the underlying abnormality in EFA biochemistry, producing a substantial clinical improvement for many atopic eczema patients.⁶⁻⁹

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EPOGAM, when added to conventional symptomatic therapy, improves all aspects of atopic eczema and substantially reduces the long-term requirement for topical steroids and antihistamines.⁶⁻⁹ EPOGAM is particularly effective in relieving itch.

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LEADERS IN RESEARCHING ESSENTIAL FATTY ACIDS

Further information is available from Scotia Pharmaceuticals Ltd, Woodbridge Meadows, Guildford, Surrey GU1 1RA. Telephone 0482 574040.

ABRIDGED PRESCRIBING INFORMATION

Presentation: Oval soft gel capsules containing 40mg gamma-linolenic acid (GLA) in evening primrose oil. **Uses:** Symptomatic atopic eczema. **Dosage:** Administration: Adults: capsules twice daily. Children: 1-12 years: 2-4 capsules daily. **Contraindications:** None known. **Warnings:** Precautions: Use with caution in patients with a history of epilepsy and known epileptogenic drugs. **Adverse Effects:** adverse effects have been reported: nausea, indigestion, headaches have occurred.

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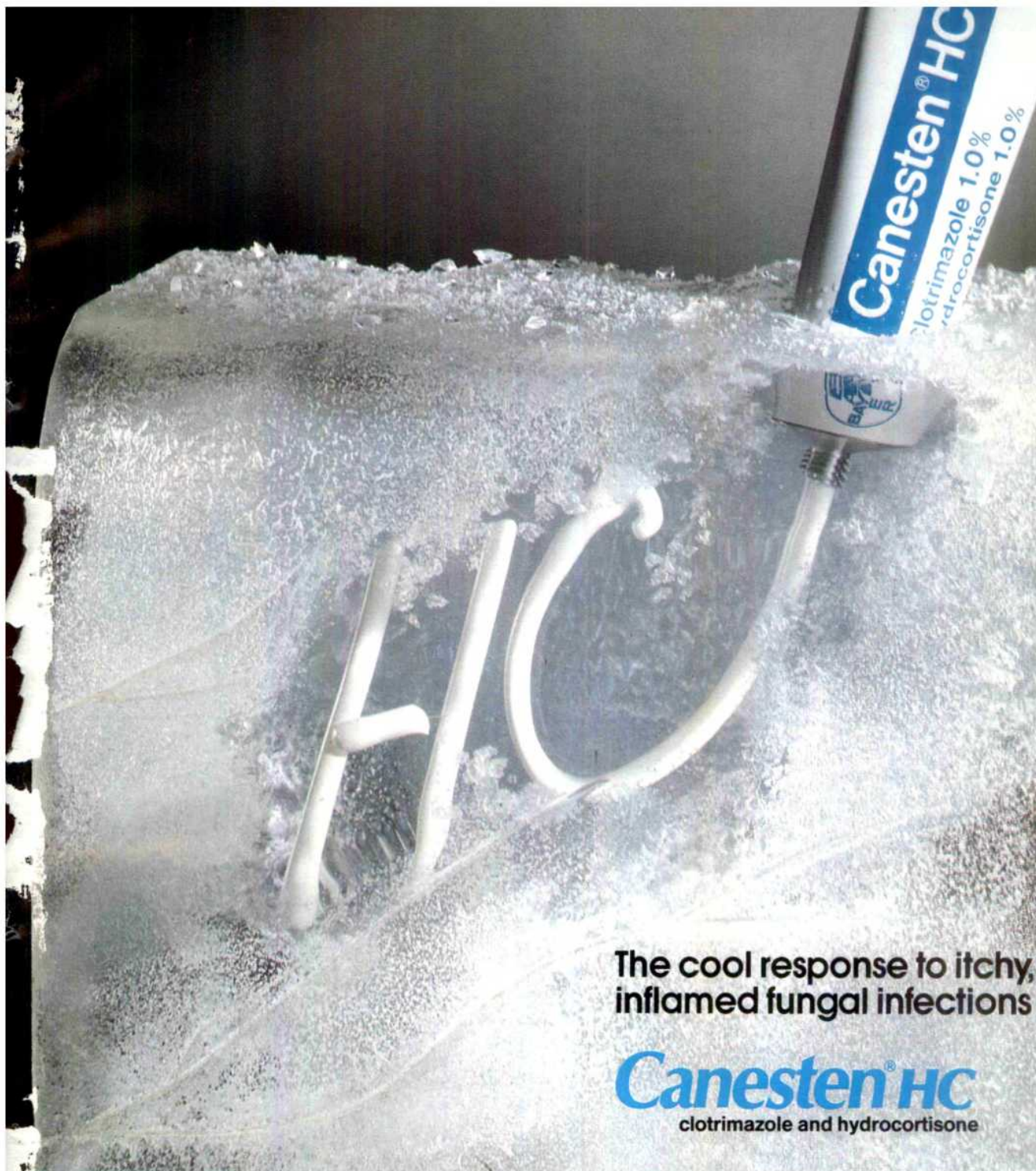
Basic NHS
240 capsules £

Product Licence No
and Number: Scotia Pharmaceuticals Ltd, 4382/0005.

REFERENCES

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9. Morse PF et al In Press.

Epogam, Scotia and EFA are registered trademarks.



The cool response to itchy,
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Canesten® HC
clotrimazole and hydrocortisone

CRIBING INFORMATION **Presentation** Canesten HC is available as a white cream containing 1.0% Clotrimazole BP and 1.0% Hydrocortisone PhEur. **Uses** The treatment of skin infections due to dermatophytes, yeasts, moulds and other fungi, where co-existing symptoms of inflammation e.g. itching, require rapid relief. **Dosage and administration** Canesten HC should be thinly and evenly applied to the affected area twice daily and rubbed in gently. **Contra-indications** Hypersensitivity to any of the ingredients. **Warnings and Precautions** As with all corticosteroids, long term continuous therapy to extensive areas of skin should be avoided, particularly in infants and children. In infants, a plaster may act as an occlusive dressing and increase absorption. **Side effects** Rarely patients may experience local mild burning or irritation immediately after applying the cream. Very rarely, the patient may find this irritation intolerable and stop treatment. Hypersensitivity reaction may occur. **Use in pregnancy** Topical administration of corticosteroids to pregnant animals can cause abnormalities of foetal development. The relevance of this to humans has not been established. In animal studies clotrimazole has been associated with teratogenic effects but following oral administration of high doses to rats there was evidence of foetotoxicity. The relevance of this effect to topical use in humans is not known. However, clotrimazole has been used in pregnant women for over a decade without attributable adverse effects. It is therefore recommended that Canesten HC should be used in pregnancy only when considered necessary by a clinician. **Accidental oral ingestion** In the event, routine measures such as gastric lavage should be performed as soon as possible after ingestion. **Pharmaceutical precautions** Store in a cool place. **Legal Category** POM. **Basic NHS Price** £3.18 per 30 g. **Product Licence Number** PL0010/0120.

Baypharm 

Further information available from:
Bayer UK Limited,
Pharmaceutical Business Group,
Bayer House, Strawberry Hill,
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REGAINE TOPICAL SOLUTION 2% ▼ Abbreviated Data Sheet Copy

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Presentation: Clear, colourless to light yellow liquid for topical application. **Uses:** Regaine is indicated for the treatment of pattern baldness in males (alopecia androgenetica). **Dosage and Administration:** Apply 1 ml twice daily to the centre of the affected area of the scalp. The total daily dose should not exceed 2ml. The method of application varies according to the disposable applicator used. In all cases the hair and scalp should be thoroughly dry, and the Regaine solution allowed to dry without the use of a hair drier. Twice daily application for 4 months or more may be required before evidence of hair growth stimulation can be expected. Onset and degree may be variable. Relapse to pre-treatment appearance following discontinuation of medication has been anecdotally reported to occur within 3-4 months. Patients should discontinue treatment if there is no improvement after one year. **Contra-Indications,**

Warnings, etc: *Contra-indications:* hypersensitivity to any of the components of the preparation. *Warnings:* Regaine is for external use only. Use only as directed. Do not apply to areas of the body other than the scalp. Use of Regaine results in slight absorption (an average of 1.4% of the applied topical dose) of minoxidil from the skin and the potential for systemic effects should be considered. The most frequently reported adverse effects have been minor dermatological reactions. *Precautions:* Patients with hypertension should be monitored closely when treated with Regaine. Regaine contains an alcohol base which will cause burning and irritation to the eye. The safety and effectiveness of Regaine in patients under 18 or over 65 has not been established. As for other topical preparations, concomitant damage or disease of the skin may lead to increased absorption of minoxidil. Regaine should not be used in conjunction with other topical agents. **Package**

Quantities: Bottles of 60 ml with one or more of the following disposable applicators: pump spray, extended tip, or rub-on assemblies. **Product Licence Number:** PL 0032/0136. Upjohn Limited, Fleming Way, Crawley, West Sussex. **Date of Preparation:** February 1988. **List Price:** £20.00. (Not available at N.H.S. expense)

Full prescribing information available on request.

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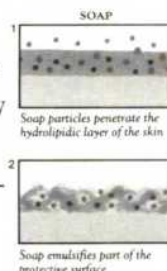


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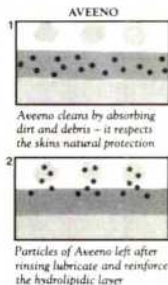
The action of soap is really quite aggressive and when it washes away the dirt it also strips away part of the skin's natural protective hydro-lipidic surface.



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The Dermatology of Bacterial Infections

G.H. Findlay

Summer 1987. 384 pages,
49 illustrations. About £50.00

The practical value of this eminently readable text is increased by the display in boxes of glossaries, epidemiology, essentials of pathology, clinical features and therapeutics. Comprehensive in its contents, it covers the field from anthrax to acne, nocardia to napkin dermatitis, rhinoscleroma to Rocky Mountain spotted fever, and tularaemia to toxic shock syndrome.

Fundamentals of Cutaneous Surgery

R.G. Bennett

Summer 1987. 864 pages,
465 illustrations.
About £59.00 *Mosby*

A highly illustrated and practical book providing dermatologists with a comprehensive guide to the procedures involved in cutaneous surgery.

Topical Steroids for Skin Disorders

M. Clement and A. du Vivier

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Uses

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Dosage and administration

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Contra-indications, warnings etc.

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Contra-indications. Skin tuberculosis, viral infections accompanied by dermal manifestations, eg. herpes simplex, vaccinia, chicken pox, measles, Syphilitic skin lesions.

Pharmaceutical precautions

Store in a cool place but do not freeze. Do not put in alloy containers.

Legal category POM

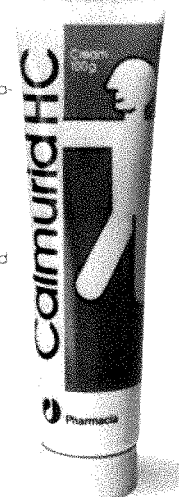
Package quantities and prices (April, 1987)

Tubes of 30 g (£2.33). Tubes of 100 g (£6.75).

Further information

Calmurid HC is a pleasant cream which does not contain lanolin or preservatives. This results in a negligible risk of allergic reactions and good patient compliance.

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Calmurid HC

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The specific interests covered include human and animal safety-testing of skin, hair and oral products, physical chemistry and technology of emulsion and dispersed systems, theory and application of surface-active agents, new developments in olfactive research, aerosol technology, and selected aspects of analytical chemistry.

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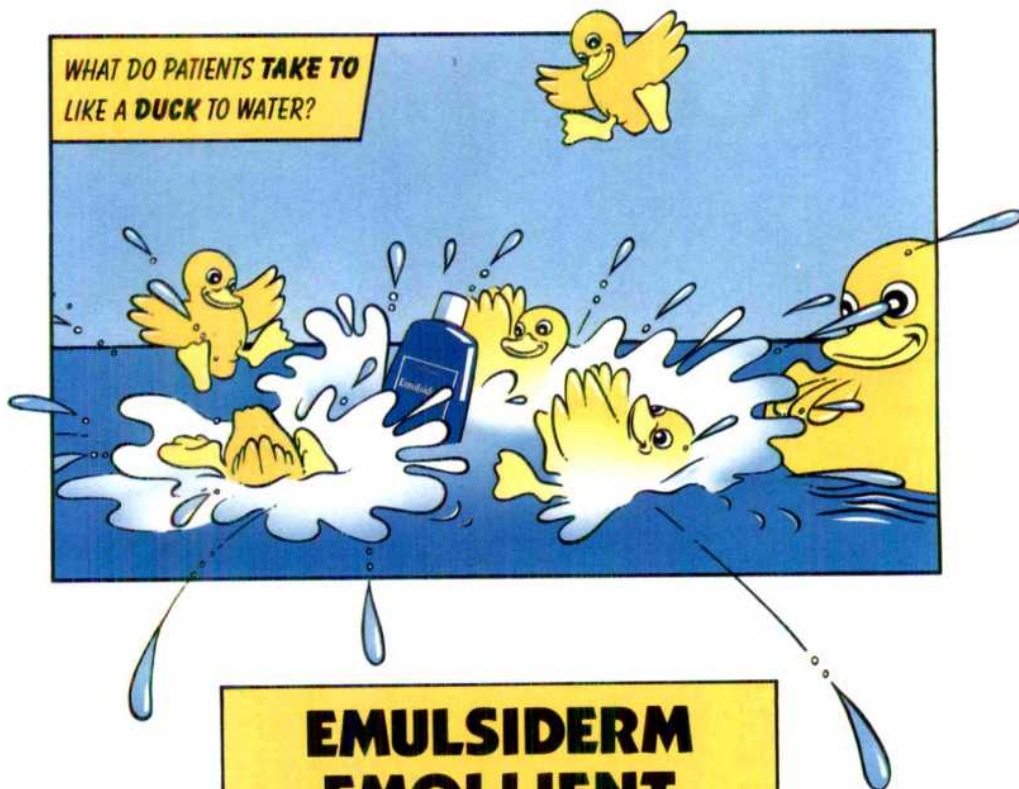
PRESCRIBING INFORMATION
Presentation Cream containing 10% urea, 5% lactic acid, in an emulsified base. **Uses** Hyperkeratosis, ichthyosis and other dry, rough scaly skin conditions. **Dosage and administration** Apply a thick layer twice daily after washing and drying. **Contra-indications, warnings etc.** Adverse Effects. Calmurid can cause smarting temporarily when applied to raw or fissured areas. **Pharmaceutical precautions** Store in a cool place but do not freeze. Do not put in alloy containers. **Legal category** G.S.L. **Package quantities and prices (March 1988)** Tubes of 50g (£1.76), Tubes of 100g (£3.49), Pump Dispenser 400g (£12.50). **Further information** Calmurid is a pleasant cream which does not contain lanolin or preservatives. This results in a negligible risk of allergic reactions and good patient compliance. **Product licence number** 0009/5009

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Further information is available on request Dermal Laboratories Limited, Gosmore, Hitchin, Hertfordshire SG4 7DR

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Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation

S. LISBY,* E. RALFKIAER,† R. ROTHLEIN‡ AND G. L. VEJLSGAARD*

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Accepted for publication 31 October 1988

SUMMARY

The presence of intercellular adhesion molecule-1 (ICAM-1) on keratinocytes of psoriatic skin lesions before and during 8-methoxypsoralen and UVA light (PUVA) treatment was studied. ICAM-1 was expressed on the keratinocytes in biopsies of the skin lesions of five patients with psoriasis. The patients who responded to PUVA treatment had a concurrent reduction of ICAM-1 expression on the keratinocytes with a reduction of the number of cells in the mononuclear cellular infiltrate and a lessening of the severity of the disease. Patients who went into remission during therapy and then relapsed showed an increase in ICAM-1 expression on keratinocytes with an increase in the number of cells in the mononuclear cell infiltrate and an increase in the severity of the disease. HLA-DR expression on keratinocytes was variable during treatment and showed no strong correlation with disease severity.

Leukocyte adhesion is a mandatory event in the pathogenesis of an inflammatory response of the skin. Leukocytes must adhere to the endothelium in order to migrate to the inflammatory lesion.¹ Lymphocytes must also adhere to antigen presenting cells^{2,3} and antigen bearing target cells so that normal immunological responses can occur.⁴ The CD18 complex of leukocyte membrane proteins^{5–7} has been identified as mediating, at least in part, leukocyte adhesion.⁸ Monoclonal antibodies to members of the CD18 complex inhibit many adhesion-dependent functions of leukocytes including: their binding to endothelium,⁹ homotypic adhesions,¹⁰ antigen and mitogen induced proliferation of lymphocytes,⁵ antibody formation¹¹ and effector functions of all leukocytes such as lytic activity of cytotoxic T cells,¹² macrophages,¹³ and all cells involved in antibody-dependent cellular cytotoxicity reactions.¹⁴ In all of the above functions, the antibodies inhibit the ability of the leukocyte to adhere to the appropriate cellular substrate which in turn inhibits the final outcome. A group of patients lacking the CD18

complex on their leukocytes have been identified¹⁵⁻²¹, and they have defective defence system responses, including the inability of most leukocytes to infiltrate into inflammatory skin lesions.

Recently ICAM-1 has been identified as a binding ligand of the CD18 complex on leukocytes.^{22,23} ICAM-1 is a glycoprotein with a molecular weight of 90-120 kd depending on the cell type from which it was isolated.²²⁻²⁴ It is inducible on fibroblasts and endothelial cells *in vitro* by inflammatory mediators such as IL-1, gamma interferon and tumour necrosis factor.^{24,25} Recently our laboratories have shown that ICAM-1 is present on keratinocytes from biopsies of skin lesions from various dermatological disorders.²⁶

We have also found that ICAM-1 is induced on lesions from allergic patch tests, whereas the keratinocytes in irritant patch test lesions failed to express ICAM-1.²⁶

In this report, the pattern of ICAM-1 expression in skin lesions of psoriasis patients were studied before and after treatment with 8-methoxypsoralen and UVA light (PUVA).

METHODS

Biopsy specimens

Biopsies were obtained from five patients with classical psoriasis. The skin biopsies were taken sequentially before and during the course of PUVA treatment. PUVA was given three to four times weekly. The biopsies were taken from the periphery of the psoriatic plaques in five patients and, in addition, biopsies were taken from the clinically normal skin in four of the patients.

Processing of specimens

The fresh skin biopsy specimens were frozen and stored in liquid nitrogen. Six micron cryostat sections were air-dried overnight at room temperature, fixed in acetone for 10 min and either stained immediately or wrapped in aluminium foil and stored at -80°C until stained.

Antibodies and histochemical reagents

Monoclonal antibodies against HLA-DR were purchased from Becton Dickinson (Mountainview, California, U.S.A.). Monoclonal anti-ICAM-1 antibody (R6-5-D6) was developed at Boehringer Ingelheim.²⁶ Peroxidase-conjugated rabbit anti-mouse-Ig and peroxidase-conjugated swine anti-rabbit-Ig were purchased from DAKOPATTS, Copenhagen, Denmark. Diaminobenzidine-tetrahydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.).

Immunoenzymatic staining procedures

Sections were incubated with monoclonal antibodies and stained using a three stage immunoperoxidase method,²⁷ with a diaminobenzidine H₂O₂ substrate. Tonsils and lymph nodes were used as positive control for anti-ICAM-1 and HLA-DR staining. Tissue stained in the absence of primary antibody were the negative controls.

RESULTS

Expression of ICAM-1 on psoriatic keratinocytes before and after PUVA treatment

The expression of ICAM-1 in skin biopsies from five patients with psoriasis were studied before and at intervals during the course of PUVA treatment. The endothelial cells in some blood vessels express ICAM-1 in both diseased and normal skin, but the intensity of staining and the number of blood vessels expressing ICAM-1 was increased in the psoriatic skin lesions. The

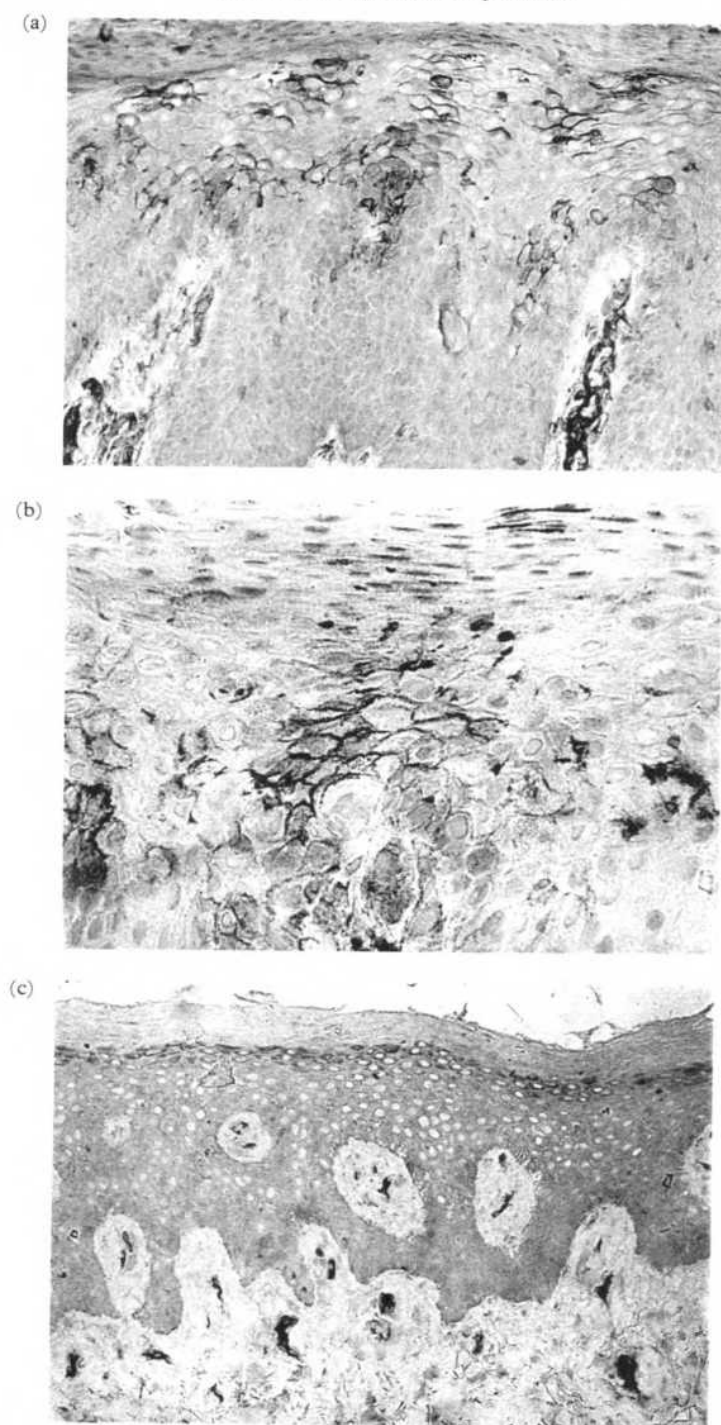


FIGURE 1. ICAM-1 expression by keratinocytes in untreated psoriatic skin (a) ($\times 150$) shown at a higher magnification in (b) ($\times 360$). Note that endothelial cells and dermal mononuclear cells are also ICAM-1-positive. In clinically normal skin (c) ($\times 150$), ICAM-1 expression is confined to endothelial cells with no labelling seen of keratinocytes.

TABLE 1. Sequential ICAM-1 expression by keratinocytes in psoriatic skin lesions (PS) and clinically normal skin (N) before and during PUVA treatment

Time before and during PUVA treatment	Patient no.									
	1 PS	2 PS	N	3 PS	N	4 PS	N	5 PS	N	
0	+	+	-	++	-	++	-	+++	-	
1 day	+									
1 week	+	+	-	-	-	++	-	+	-	
2 weeks	+++†			+	-	+	-	+	-	
3 weeks	++									
4 weeks	++	+	-	-*	-	+++†	-			
5-6 weeks		-*	-					-*	-	
7 weeks						(+)	(+)	+++†	-	
10 weeks	(+)*							-*	-	

+++ Many positive keratinocytes.

++ A proportion of positive keratinocytes.

+ Focal positive keratinocytes.

(+) Very few scattered positive keratinocytes.

- No positive staining.

* Clinical remission.

† Clinical relapse.

pattern of expression of ICAM-1 in keratinocytes of untreated psoriatic skin lesions from the five patients varied from only small clusters of cells to many keratinocytes that were stained (Fig. 1a, b). During the course of PUVA treatment, the ICAM-1 expression in the biopsies from two of the patients (patients 2 and 3) showed a marked reduction that preceded or was concurrent with clinical improvement (Table 1). Patients 1, 4 and 5 showed changes in ICAM-1 expression during the PUVA treatment that were related to improvement and relapse. There was no ICAM-1 expression on the keratinocytes from normal skin both before and after PUVA treatment. This indicates that PUVA does not induce ICAM-1 on the keratinocytes in normal skin.

Of note was the observation that the density of the mononuclear cell infiltrate correlated with the amount of ICAM-1 expression on keratinocytes; the ICAM-1 expression being reduced when there was a reduction in the number of mononuclear cells in the skin during PUVA treatment.

The expression of HLA-DR on keratinocytes was variable. However, there were no HLA-DR positive biopsies that were not also ICAM-1 positive.

DISCUSSION

ICAM-1 is inducible on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, tumour necrosis factor and gamma-interferon and occurs with the infiltration of lymphocytes into inflammatory lesions *in vivo*.²⁴ Further ICAM-1 is expressed on non-haematopoietic cells such as vascular endothelial cells, thymic and other epithelial cells and on fibroblasts. It is also expressed on haematopoietic cells such as tissue macrophages, mitogen-stimulated T lymphocyte blasts, and germinal center B-cells and dendritic cells in tonsils,

lymph nodes and Peyer's patches.²⁴ Recent studies have shown that ICAM-1 is expressed on keratinocytes in benign inflammatory lesions such as allergic eczema, lichen planus, exanthems, urticaria and bullous diseases.²⁶ Skin reactions provoked by the application of a hapten on the skin to which the patient is allergic also revealed a heavy ICAM-1 expression on the keratinocytes. On the other hand primary irritant reactions of the skin did not reveal ICAM-1 expression on the keratinocytes.²⁶ In the present study we have looked for ICAM-1 expression in psoriatic lesions before and after PUVA treatment. Before treatment, ICAM-1 expression was pronounced on the keratinocytes correlated with the dense mononuclear cellular infiltrate. During PUVA treatment there was a marked decrease of the ICAM-1 staining which paralleled the clinical improvement. Histologically the dermal infiltrate also diminished. With clinical relapse, the expression of ICAM-1 on the keratinocytes was increased, as was the density of the dermal infiltrate. The expression of ICAM-1 on keratinocytes corresponded to the density of the mononuclear cellular infiltrate in the dermis. What is not clear is whether the expression of ICAM-1 on keratinocytes is initiating the dermal inflammatory infiltrate or is the result of this response. It is known that inflammatory mediators such as gamma-interferon, IL-1, and/or TNF will induce ICAM-1 on endothelial cells and fibroblasts. Since these mediators are capable of being secreted by cells of the dermal infiltrate in psoriatic lesions, it is possible that they will locally induce ICAM-1. On the other hand, it is possible that the dermal infiltrate and clinical response is due, in part, to ICAM-1 expression on the keratinocytes allowing the dermal infiltrate to develop. This is supported by data presented here which shows that clinical response to PUVA treatment resulted in a pronounced decrease of ICAM-1 expression on keratinocytes parallel to a more moderate decline of the mononuclear cells. This suggests that ICAM-1 expression on keratinocytes is responsible for initiating and maintaining the dermal infiltrate and that PUVA treatment may down regulate ICAM-1 which in turn mitigates the dermal infiltrate. The data also indicates that there was variable HLA-DR expression on keratinocytes during PUVA treatment. However, in no instance was there HLA-DR expressions on keratinocyte in biopsies where the keratinocytes did not express ICAM-1. This confirms previous findings showing that all HLA-DR biopsies from benign cutaneous disorders also expressed ICAM-1.²⁶

In conclusion, the expression of ICAM-1 on keratinocytes of psoriatic lesions correlates with the clinical severity of the lesion as well as with the size of the dermal infiltrate. Thus, ICAM-1 may play a central role in psoriasis and inhibition of its expression and/or inhibition of its interaction with the CD18 complex on mononuclear cells may act as a treatment of the disease.

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A flow cytometric study of Langerhans cell histiocytosis

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SUMMARY

Langerhans cell histiocytosis (LCH), or histiocytosis X, is now generally considered to be a non-malignant condition. A flow cytometric (FCM) study of a single case has, however, been published which claimed to provide evidence to contradict this. The presence of DNA-ploidy as detected using this technique is a feature of malignant and pre-malignant disease. In this reported single case, DNA-ploidy was present but the clinical features of this patient were atypical for LCH. We have performed a FCM study of the DNA of nine biopsies of LCH lesions from six patients with well-established disease. In addition, in one of these, fresh tissue studies including the use of an anti-CD1 monoclonal antibody to specifically label the LCH cells were performed. In all cases the DNA content of the cells was entirely normal. We therefore found no evidence that LCH is a neoplastic disorder.

The nature of LCH, benign, reactive or malignant, has been a controversial issue. Most clinicians dealing with patients with LCH now regard it as a non-malignant disease¹ although this is not supported by any scientific evidence. Goldberg *et al.* recently reported a case of LCH in a 76-year-old man with single system disease of the skin consisting of numerous cutaneous nodules.² A biopsy specimen of the skin revealed an infiltration of histiocytes which were found to express CD1 on the cell surface and to contain the S100 protein in their cytoplasm, whilst on electron microscopy Birbeck granules were seen. Analysis of the DNA content by FCM revealed an aneuploid peak in addition to the normal diploid one, suggesting that this patient had a malignant condition.

In addressing this issue we performed FCM on nine formalin-fixed, paraffin-embedded biopsies of LCH lesions from six patients with well-established disease. In one of these six patients additional studies of the DNA from a fresh lesion were performed. In this one case the

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LCH cells were labelled with the anti-CD1 antibody OKT6 and the DNA content of these cells was examined specifically.

METHODS

Biopsy material

Formalin-fixed, paraffin-embedded material was obtained from nine patients with clinically well-established Langerhans cell histiocytosis (see Table 1). Sections were taken from each block and were stained using haematoxylin and eosin and for S100 protein. Only those blocks with a substantial infiltration of S100-positive LCH cells were used. One sample was post-mortem material and was autolysed. Two samples were processed but insufficient cells were available for examination. Results were available from the remaining six patients.

In addition, fresh material was obtained from a gingival biopsy of one of the above six patients. This specimen was divided into two. One half was embedded in OCT embedding medium and was snap-frozen in liquid nitrogen. A cryostat was used to cut 5 µm thick sections which were air-dried and then stained using an anti-CD1 monoclonal antibody, OKT6 (Ortho, Rariton, NJ, U.S.A.) by an APAAP technique.³ This showed that the dermis was infiltrated with characteristic LCH cells which were stained by the CD1 antibody. The second half was placed in antibiotic-containing RPMI tissue culture medium (Gibco, Uxbridge, U.K.) at 4°C and was processed for FCM within 2 h.

Flow cytometry

Paraffin-embedded material. Cells were prepared for RCM using a method described by Hedley et al.⁴ and Newton, Camplejohn and McGibbon.⁵ For the skin biopsies five 60 µm thick sections were used, and for the other specimens a single 60 µm section was used. The sections were dewaxed before incubation in pepsin 5 mg/ml at 37°C for 30 min at pH 1.5. After centrifugation and resuspension of cell nuclei in Isoton (Coulter Electronics, Luton, U.K.), the nuclei were stained with 4,6 diamidino, 2, phenylindole hydrochloride (DAPI) (Boehringer, Mannheim, West Germany). This is considered to be a DNA-specific stain. Cells prepared in

TABLE 1. Patients from whom material was obtained

Case no.	Sex	Age	Site of biopsy	Systems involved
1	F	54	mouth, ear	Bone, skin, alveolar ridge.
2	F	4	lymph node	Lung, lymph nodes, bone marrow, skin, spleen. Died of disease.
3	M	2	lymph node	Liver, lymph nodes.
4	M	2	pituitary, lymph node × 2	Skin, lungs, pituitary, liver, lymph nodes. Died of pneumonia.
5	F	19	skin	Skin.
6	F	6/52	skin	Bone, skin.
7*	M	1	lymph node	Lymph node, skin, spleen, pituitary. Died of disease.
8*	F	26	skin	Skin, pituitary.
9*	M	22	skin	Skin.

* Cases for which results not obtained.

this way were subjected to FCM. The coefficient of variation (CV) of the peak of normal diploid cells was used to assess the quality of FCM results.

Fresh material. The tissue was cut into 1 mm wide strips and was incubated in collagenase (0.5 mg/ml) (BDH, Poole, U.K.) and dithioerythrol (31 mg/20 ml) (BDH) in MEM (Gibco) at 37°C for 30 min. The epidermis was then peeled off the dermis using watchmakers forceps to facilitate disaggregation and both were then separately incubated in trypsin (0.5 mg/ml) (Cooper Biomedical) in versene (Gibco) at 37°C for 10 min.

The resultant cell suspensions were passed through a 19 and then 21 gauge needle to disaggregate the cells. The dermal and epidermal cells were then pooled, as there were insufficient numbers of cells to allow separate analysis, and filtered through 35 µm pore polyester gauze. The cells were then incubated with the anti-CD1 antibody (10 µl/10⁶ cells) for 30 min. The cells were washed in MEM and resuspended in MEM with 5% human serum containing a (Fab)₂ biotinylated rabbit-anti-mouse antibody (Dako, High Wycombe, U.K.). The cells were incubated on ice for 30 min, washed, then resuspended in medium containing avidin-FITC (Dako). After a further incubation on ice for 20 min the cells were washed and then fixed in cold 70% ethanol. After fixation the cells were stained with propidium iodide with RNA-ase. Simultaneous measurement of DNA content (red fluorescence), antibody fluorescence (green) and Coulter volume were made using a Becton Dickinson FACS analyser and the data stored in list mode on a Consort 30 computer. DNA content was measured on a linear scale, while green fluorescence and volume were measured using a 3 decade log amplifier. 10,000 cells were measured per sample.

RESULTS

All DNA histograms of the six patients were diploid. The mean coefficient of variation of the G1 peak was 5.4%. The CD1-positive cells from the fresh sample, shown on frozen tissue section to be the LCH cells in the dermis and occasional Langerhans cells in the epidermis, comprised 4% of the total number of cells obtained from the specimen and were also diploid (Fig. 1).

Due to the small size of the fresh biopsy, it was not possible to perform flow cytometry on the dermis and epidermis separately. However, previous experience has shown⁶ that, using Coulter volume, it is possible to make a good distinction between most dermal cells and keratinocytes. Figure 2(a) shows a plot of DNA versus volume. A population of small non-proliferative cells can be seen (population A, Fig. 2(a)), and a distinct band of proliferative keratinocytes is apparent (population B) with G₁, S and G₂ DNA contents. A population of still larger cells (C) is present. These cells can be extremely large, non-proliferative and show a degree of non-specific fluorescence; they are differentiated keratinocytes. In Figure 2(b), volume is plotted against green fluorescence for the control sample stained in the absence of OKT6 antibody. Dermal cells (A) and keratinocytes (B) can be distinguished. An identical plot is given for the anti-CD1 stained sample (Fig. 2(c)) and in this a small additional population of antibody positive cells can be seen. These cells have a volume equivalent to the proliferative keratinocytes (Fig. 2a, population B) and cannot be distinguished from them without the use of anti-CD1 antibody. From Figure 2(c), by analogy with earlier work,^{5,6} it can be suggested that 30% of the total cells are non-proliferative dermal element, 66% are keratinocytes and 4% are CD1-positive LCH cells and Langerhans cells.

Figure 3 is an overlap histogram showing fluorescence intensity in the control and OKT6 stained samples excluding putative keratinocytes (population B in Fig. 2(c)).

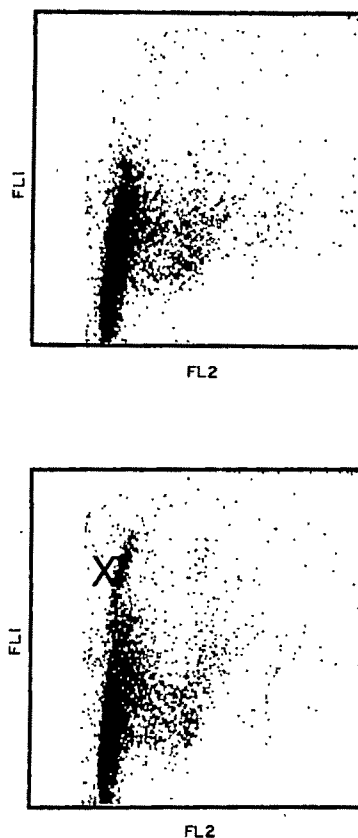


FIGURE 1. Two scatter diagrams are seen. The x axis (FL2) on a linear scale represents the DNA content of the cells. The y axis (FL1), on a 3-decade log scale represents the intensity of fluorescence emitted by OKT6 labelled cells. The upper figure is the control scattergram; cells stained for DNA only. Examination of the lower figure shows a population of cells (X) which are the OKT6 positive cells absent from the control scattergram alone. These represent 4% of the total number of cells.

DISCUSSION

Following a meeting of the Histiocyte Society in 1985 it was suggested that the term Langerhans cell histiocytosis be used to replace the designations Hand-Schuller-Christian disease, Letterer-Siwe disease, eosinophilic granuloma and acute and chronic histiocytosis X.⁷ For many years Letterer-Siwe disease was thought to behave in a similar manner to malignancies and to be invariably fatal.⁸ However, the spontaneous resolution of most cases of single-system LCH, and the occasional spontaneous regression of cases with multisystem disease,⁹ has led most clinicians to regard LCH as a non-malignant disorder. In a recent reclassification of the histiocytic disorders,¹⁰ LCH was classified into a group of its own, separate from the malignant histiocytic conditions and separate from malignant conditions of Langerhans cells.

DNA aneuploidy has been demonstrated in 80-90% of solid tumours¹¹ and also in a number of premalignant conditions such as chronic atrophic gastritis¹² and lichen sclerosis et atrophicus.⁵ Except for tetraploid stemlines, normal or reactive tissue is not associated with

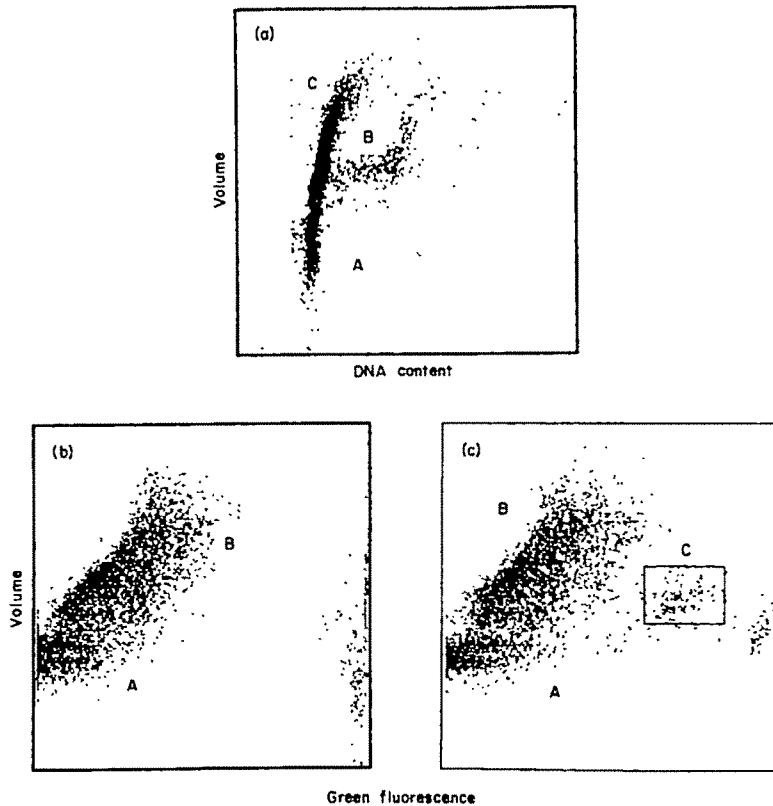


FIGURE 2. (a) Plot of DNA content on the x axis versus Coulter volume on the y axis. Volume is plotted on a 3-decade log scale. Population A is small, non-proliferative putative dermal cells, whilst population B consists of proliferative keratinocytes and population C is very large differentiated keratinocytes. (b) and (c) Plots of green (OKT6) fluorescence versus volume. Both parameters are plotted on a 3-decade log scale. Population A represents small putative dermal cells and population B consists of larger predominantly keratinocyte cells. There is a non-specific increase in fluorescence with cell size in this population. (b) represents the control sample and (c) the results for the OKT6 sample. In (c) a distinct small population (population C) of OKT6-positive cells is apparent. These cells are larger than dermal cells and have a volume corresponding to proliferative keratinocytes.

aneuploidy¹¹ so that aneuploidy is thought to be implicit of neoplasia.¹³ The finding of a diploid population of cells does not prove a benign state as a malignant tumour may have diploid DNA-ploidy. Furthermore, FCM may miss ploidy aberrations of up to three chromosomes.¹⁴

Goldberg *et al.* recently reported a flow cytometric study of a single case of LCH in which DNA-ploidy was demonstrated.² It was suggested, therefore, that this provided evidence that LCH may be a malignant process. However, this was a single report and the presentation of the disease was atypical. The patient was elderly, i.e. 76-years-old and presented with generalized skin nodules of 0.5–1.5 cm diameter. Over 50% of patients with LCH are children under the age of 15¹⁵ and LCH in the elderly is unusual. LCH of the skin is most often maculopapular or seborrhoeic eczema-like in appearance although a solitary nodular plaque may occur.¹⁵ The presence of generalized nodules between 0.5 and 1.5 cm in diameter is more suggestive of

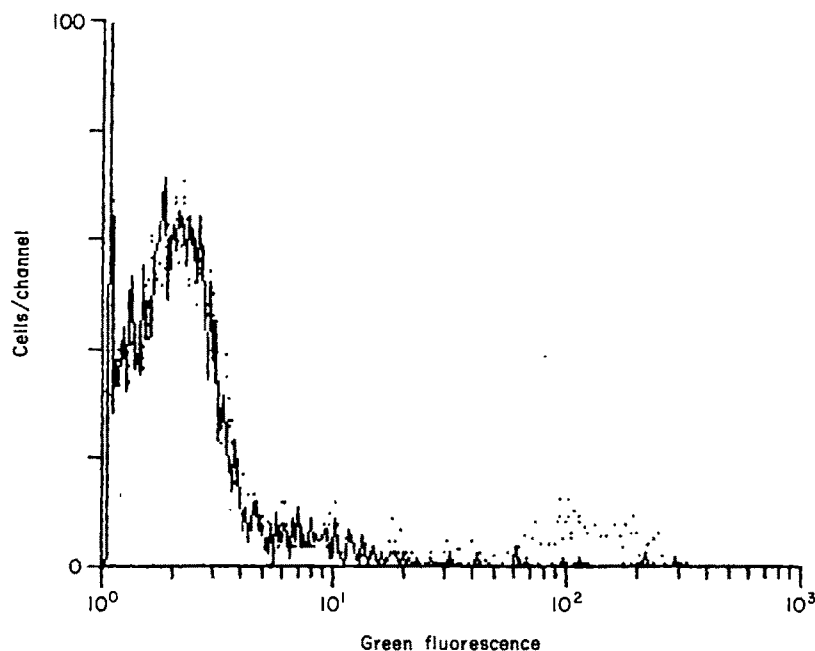


FIGURE 3. A fluorescence histogram of green fluorescence (OKT6 linked fluorescence). The solid line represents a control sample processed without addition of the primary OKT6 monoclonal antibody. The dotted line represents the result for the OKT6 stained sample. A clear positively stained population is apparent in this sample.

malignant histiocytosis or progressive nodular histiocytosis. In malignant histiocytosis the histiocytes may have the S100 protein and Birbeck granules present in their cytoplasm¹⁶⁻¹⁸ and rare Langerhans cell malignant histiocytoses have been recognized.¹⁰ Both malignant histiocytosis and progressive nodular histiocytosis may have normal, reactive Langerhans cells, which are S100, Birbeck granule and CD1-positive, present in the lesions which may cause diagnostic confusion.¹⁹ It is possible that the case reported by Goldberg *et al.* would be better classified as one of the non-Langerhans cell histiocytoses or as malignant Langerhans cell histiocytosis. It is also not certain that the aneuploid cells reported were the histiocytes; other cells in the skin of the elderly patient may be responsible for this aneuploid subpopulation of cells.

We report a study of six more typical cases of LCH, all with well-established disease. Five of them had proven multisystem LCH. Flow cytometric analysis of fixed tissue from all these patients showed a normal diploid DNA content. The examination of OKT6-labelled fresh tissue from one of these enabled us to examine the DNA content of the putative LCH cells more specifically and these cells were also shown to be diploid. We have thus failed to confirm the findings of Goldberg *et al.*, and have found no evidence to support the suggestion that LCH is a malignant process. This is in accordance with the current view that LCH is distinct from the malignant histiocytoses.

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Suppression of contact sensitivity by local hyperthermia treatment due to reduced Langerhans cell population in mice

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SUMMARY

The effects of local hyperthermia treatment on contact sensitivity (CS) and on the number of Langerhans cells (LCs) were studied in mice. CS was significantly suppressed when mice were sensitized in the hyperthermia treated skin 1, 2 or 4 days after treatment (43°C for 45 min). This suppressive effect was not observed 7 or 14 days after the treatment. CS was also suppressed when mice were sensitized in non-treated skin 1 day after the treatment. The density of LCs detected as ATPase-positive cells also decreased significantly 1, 2, 4 and 7 days after the treatment. There appeared to be a positive correlation between the number of LCs and the extent of CS when mice were sensitized at hyperthermia treated skin. It was observed that this suppressive effect on CS was dose- and temperature-dependent. It could be transferred by spleen cells from the hyperthermia treated and DNFB-sensitized donors, and was antigen specific when spleen cells were transferred before sensitization of the recipient mice. This indicated it was, in part, associated with the induction of suppressor cells. These findings suggest that local hyperthermia treatment reduces the number of LCs with subsequent suppression of the induction phase of delayed-type hypersensitivity by the generation of antigen-specific suppressor cells.

Recently, there have been several reports on the effectiveness of whole-body and local hyperthermia treatments for cancer, both in experimental animals and patients.¹⁻³ The importance of hyperthermia treatment in managing cancer has been increasing with the improvement of equipment as well as the continuing investigation of the mechanisms of hyperthermia treatment. However, hyperthermia treatment may have unfavourable systemic effects, especially on the host's immune system. It has been reported that the function of murine cytotoxic T lymphocytes was inactivated after exposure to *in vitro* hyperthermia,^{4,5} and that murine lymphocyte viability decreased after *in vitro* hyperthermia.⁶ In addition, *in vitro*

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hyperthermia suppressed phytohaemagglutinin (PHA)-induced lymphocyte blastogenesis.⁶ Human delayed skin reactions to purified protein derivatives of tuberculin (PPD) and PHA after whole body hyperthermia also decreased.⁷ Thus, in order to use this treatment more effectively for cancer therapy, we must increase knowledge about the unfavourable effects of hyperthermia. In our research, we examined the suppressive effects of a single local exposure to hyperthermia on contact sensitivity (CS) to 1-fluoro-2,4-dinitrobenzene (DNFB) and its mechanism with special reference to Langerhans cells (LCs).

METHODS

Animals

Albino female ICR mice, 8–10 weeks old, were used, except for the transfer study, where female BALB/c mice, 8–10 weeks old, were used. All mice used in experiments were obtained from Shizuoka Experimental Animal Corporation, Hamamatsu, Japan, and were rested for at least 2 weeks before use.

Local hyperthermia treatment

Local hyperthermia treatment was given according to the method of Miyakoshi *et al.*,^{8,9} with slight modification. As shown in Figure 1, each unanaesthetized mouse was put into a special cage. The left hind leg was strapped down with surgical tape, and a 50 g weight was attached to the right hind leg with an adhesive (Toa Chemical Co., Tokyo, Japan) to prevent the mouse from withdrawing it when put into hot water. It was then put in a circulating water bath (ET-35P; Toyo Seisakusho, Tokyo, Japan), in which the temperature of the hot water was maintained constant within the desired temperature $\pm 0.05^{\circ}\text{C}$.

During hyperthermia treatment, a current of cool air was blown across the bath to prevent the body temperature from rising and to reduce the humidity of the inspired air. In our experiments, a hyperthermia treatment at 43°C for 45 min was given to each mouse, except for those experiments to determine the temperature- and dose-dependent effect of hyperthermia treatment.

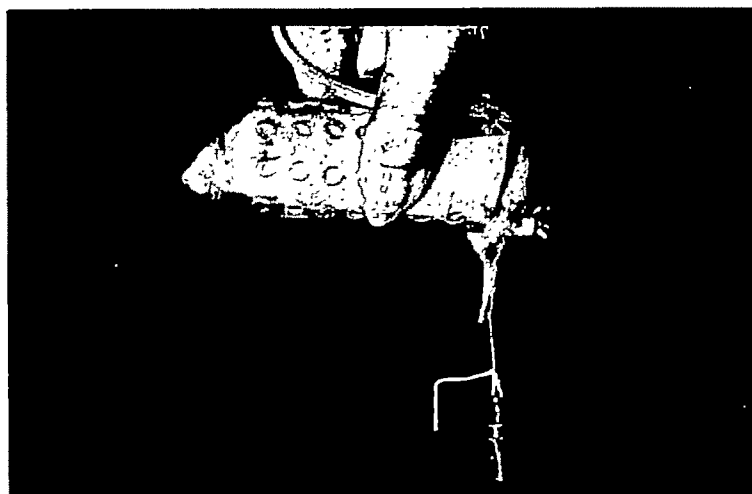


FIGURE 1. Procedure of hyperthermia.

Chemicals

DNFB (Nakarai Chemical Co., Kyoto, Japan) was dissolved in an olive-acetone mixture (1:4), and used for sensitization and elicitation of CS. Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) was dissolved in ethanol for sensitization and in olive-ethanol mixture (2:1) for elicitation of CS.

Sensitization and elicitation of CS

For sensitization, a solution of 50 μ l of 0.5% DNFB or 100 μ l of 3% oxazolone was applied epicutaneously on the shaved skin of the right hind leg (hyperthermia treated skin) or the left hind leg (non-treated skin). Five days after the sensitization, the animals were challenged on both sides of the ear lobe with painting of 20 μ l of 0.2% DNFB or 1% oxazolone. Before elicitation, the basal line thickness of both ears was measured with a dial thickness gauge (Peacock, Tokyo, Japan). Ear thickness was measured 24 h after ear challenge and was expressed as the mean increment in thickness above the basal line control values.

Staining for LCs

LCs were stained with the method of Mackenzie and Squier,¹⁰ as modified by Juhlin and Shelley.¹¹ The epidermis was separated from the underlying dermis by incubation in buffered EDTA for 2 h at 37°C. The separated epidermis was then placed in a normal saline solution for 30 min at room temperature and then immersed in a cacodylate-formaldehyde solution for 20 min at 4°C. After rinsing in distilled water, the epidermis was processed in an ATP-Pb solution at 37°C for 15 min and in an ammonium sulphide solution for 20 min at room temperature. After the final wash in distilled water, the specimen was mounted in glycerine jelly. The number of LCs within an area of 0.04 mm² was counted by examining the ATPase positive cells in the specimens at a high power resolution ($\times 400$) and was expressed as cells/mm².¹²

Cell transfer

Cell transfer study was performed to demonstrate if the suppressive effect of hyperthermia was transferable and if the suppression was afferent or efferent.

(i) afferent phase study: donor BALB/c mice were treated with hyperthermia at 43°C for 45 min 1 and 2 days before sensitization. Then, they were sensitized with 0.5% DNFB at the hyperthermia treated skin. Five days after sensitization, single cell suspensions were prepared by teasing the spleen with forceps in RPMI 1640 (Nissui Pharmaceuticals, Tokyo, Japan) containing penicillin (100 U/ml) and streptomycin (100 μ g/ml). The cells were washed twice with RPMI 1640 by centrifugation at 200 *g* for 10 min at 4°C. The viable cell value, as assessed by trypan blue dye exclusion, was always greater than 95%. Each mouse received 1×10^8 cells in 0.4 ml of RPMI 1640 intravenously. One hour after cell transfer, the recipient BALB/c mouse was sensitized with 0.5% DNFB or 3% oxazolone.

(ii) efferent phase study: donor BALB/C mice were treated with hyperthermia at 43°C for 45 min 1 and 2 days before sensitization. Then, donor mice were sensitized with 0.5% DNFB at the hyperthermia treated skin. At the same time, recipient mice were sensitized with 0.5% DNFB or 3% oxazolone. Five days after sensitization, spleen cells from donor mice were injected intravenously into recipient mice. One hour after cell transfer, recipient mice were elicited with 0.2% DNFB or 1% oxazolone.

RESULTS

Suppressive effects of local hyperthermia on CS to DNFB

As shown in Figure 2, a single local hyperthermia treatment significantly suppressed the CS reaction to DNFB when the mice were sensitized on the hyperthermia treated skin 1, 2 or 4 days after hyperthermia treatment ($P < 0.005$ or $P < 0.01$). This suppressive effect was not significant 7 or 14 days after treatment, yet, suppressive trends on CS remained to some extent. From these results, it can be inferred that local hyperthermia treatment has a reversible suppressive effect on CS in mice.

Effects of hyperthermia treatment on the number of ATPase-positive cells

The density of LCs detected as ATPase-positive cells in the epidermis of hyperthermia treated and non-treated skin (positive control) was determined at various times after the treatment. The results are shown in Figure 2. The number of ATPase-positive cells in hyperthermia treated skin dramatically decreased 1, 2 and 4 days after the treatment and returned to normal after 14 days.

Correlation between the degree of CS and the number of ATPase-positive cells

As shown in Figure 2, both the number of ATPase-positive cells and the CS reaction were reduced 1, 2 and 4 days after the treatment and returned to normal after 14 days. Therefore, there seems to be a positive correlation between the number of ATPase-positive cells and the expression of CS.

Temperature-dependent suppressive effect of hyperthermia on CS

As shown in Table 1, the extent of CS decreased with increasing temperature. The local hyperthermia at 42°C or higher showed significant suppressive effects, however, suppression was not significant at 41°C. Thus, hyperthermia treatment has a temperature-dependent suppressive effect on CS.

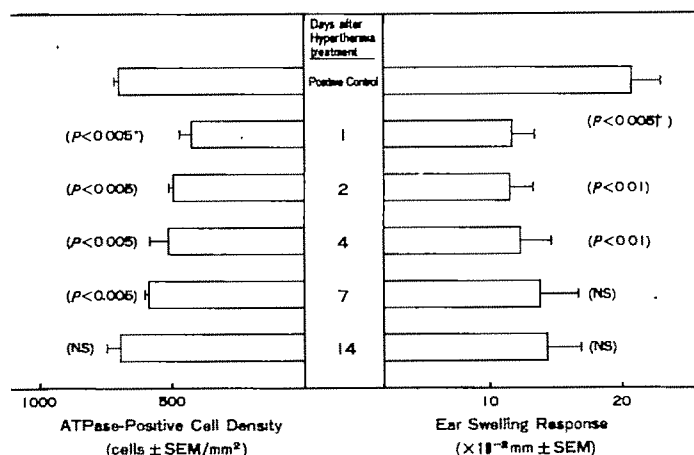


FIGURE 2. The effect of local hyperthermia on the number of ATPase-positive cell density and CS to DNFB in mice. *,†P value vs. positive control by Student's *t*-test is shown.

TABLE 1. Temperature-dependent suppressive effect of hyperthermia treatment on CS in mice

Temperature of hyperthermia* (°C)	Number of mice	Ear swelling response ($\times 10^{-2}$ mm)	P value† vs. positive control
43	5	11.6 ± 5.6 ‡	<0.01
42	5	14.8 ± 5.6	<0.05
41	5	16.9 ± 5.5	NS
37 (positive control)§	5	18.9 ± 6.8	

* Mice were sensitized with DNFB 1 day after hyperthermia treatment on hyperthermia treated skin. Local hyperthermia treatment for 45 min was given.

† P value vs. positive control by Student's *t*-test is shown. NS = not significant.

‡ Mean value $\pm$ standard deviation (SD) is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

§ In order to cancel the effect of placing mice into their apparatus and securing their legs on CS, mice which were treated at 37°C (comparable to the body temperature of normal mice) for 45 min were used as positive control.

Dose-dependent suppressive effect of hyperthermia on CS

As shown in Table 2, suppressive effects on CS were reduced by decreasing the dose. However, hyperthermia treatment at 43°C for 15 min still showed a significant effect. Suppressive effects were enhanced by repeating the treatments. As shown in Table 3, repeating the treatments three or four times showed greater suppressive effects than a single hyperthermia treatment. These data suggest that hyperthermia treatment has a dose-dependent suppressive effect on CS in mice.

TABLE 2. Effects of duration of hyperthermia treatment on CS in mice

Duration of hyperthermia*	Number of mice	Ear swelling response ($\times 10^{-2}$ mm)	P value†
0 min (positive control)‡	5	25.4 ± 6.2 §	—
15 min	5	18.9 ± 4.9	<0.05
30 min	5	14.7 ± 6.6	<0.005
45 min	5	11.7 ± 4.7	<0.005

* Local hyperthermia treatment at 43°C was given. Mice were sensitized at hyperthermia treated skin with DNFB 1 day after hyperthermia treatment.

† P value vs. positive control by Student's *t*-test is shown.

‡ Non hyperthermia treated mice were used as positive control.

§ Mean value $\pm$ SD is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

TABLE 3. Effects of repeated hyperthermia treatment on CS in mice

Number of treatments*	Number of mice	Ear swelling response ($\times 10^{-2}$ mm)	P value†
0 (positive control)	5	17.0 $\pm$ 5.2‡	—
1	5	11.0 $\pm$ 6.2	<0.025
2	5	8.7 $\pm$ 4.6	<0.005
3	5	5.6 $\pm$ 2.3	<0.005
4	5	5.3 $\pm$ 3.0	<0.005

* Hyperthermia treatment at 43°C for 45 min was given. Mice were sensitized on hyperthermia treated skin 1 day after hyperthermia treatment(s).

† P value vs. positive control by Student's *t*-test is shown.

‡ Mean value $\pm$ SD is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

Local/systemic suppressive effect of hyperthermia on CS

As shown in Figure 2 and Tables 1 and 2, it was demonstrated that hyperthermia treatment suppressed the CS reaction when mice were sensitized on hyperthermia treated skin. However, it is not clear that the inhibition is produced by a local mechanism of hyperthermia treatment. In order to elucidate whether the suppressive effect of hyperthermia is local or systemic, the following experiment was performed. Mice were treated with hyperthermia at 43°C for 45 min 1, 2 and 4 days before sensitization respectively. Then, mice were sensitized on hyperthermia treated skin (right hind leg) or on non-treated skin (left hind leg). As shown in Table 4, suppression of CS was always much greater in mice sensitized on hyperthermia treated skin than

TABLE 4. Local/systemic suppressive effect of hyperthermia treatment on CS in mice

Days after hyperthermia*	Ear swelling response ($\times 10^{-2}$ mm)			
	Sensitization on hyperthermia treated skin	P value† vs. positive control	Sensitization on non-treated skin	P value† vs. positive control
1	10.4 $\pm$ 7.1‡	<0.01	13.3 $\pm$ 5.5	<0.01
2	11.6 $\pm$ 5.4	<0.01	17.8 $\pm$ 5.9	NS
4	11.8 $\pm$ 5.2	<0.01	19.4 $\pm$ 7.4	NS
Positive control§	20.8 $\pm$ 6.7			

* Hyperthermia treatment at 43°C for 45 min was given. Mice were sensitized with 0.5% DNFB 1, 2 or 4 days after hyperthermia treatment and elicited with 0.2% DNFB 5 days after sensitization.

† Statistical analysis was performed by Student's *t*-test. NS = not significant.

‡ Mean value $\pm$ SD is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

§ Mice, which were not treated with hyperthermia, were used as positive control.

in mice sensitized on non-treated skin. This fact indicates that the inhibitory effect of hyperthermia on CS is mainly induced by a local mechanism. However, the CS reaction of mice which were sensitized on non-treated skin 1 day after hyperthermia treatment was also suppressed. This suppression was not observed when mice were sensitized 2 or 4 days after the treatment on non-treated skin. From these results, it was concluded that hyperthermia treatment had both systemic and local suppressive effects on CS. The systemic suppressive effect disappeared earlier than the local effect after hyperthermia treatment.

Transfer study

As shown in Table 5, the CS reaction of the recipient mice was remarkably suppressed when they received spleen cells from hyperthermia treated donors before sensitization and they were sensitized and elicited with DNFB. However, this suppressive effect was not observed when they were sensitized and elicited with oxazolone, suggesting an antigen-specificity of the suppressor cells. As shown in Table 6, the CS reaction of the recipient mice that received spleen cells from hyperthermia treated mouse after sensitization (before elicitation) was not suppressed when they were sensitized and elicited by either DNFB or oxazolone. These data showed that hyperthermia-induced suppressive effect on CS was transferrable by spleen cell, antigen specific and present in the afferent limb of CS, but not in the efferent limb.

DISCUSSION

In the present experiments, we have demonstrated that only a single exposure to local hyperthermia suppressed CS to DNFB in mice when they were sensitized within 4 days after hyperthermia treatment at the treated skin and 1 day after the treatment on non-treated skin. This suppressive effect was not statistically significant when the mice were sensitized 7 or 14 days after the treatment at the treated skin, or later than 1 day after the treatment on non-treated skin. These results indicate that the suppressive effect of local hyperthermia is reversible, and

TABLE 5. Transfer study of hyperthermia treatment on CS in mice—Afferent phase study*

	Hyperthermia treatment† of cell donors	Sensitization of cell donors	Sensitization and elicitation of recipients	Ear swelling response ($\times 10^{-2}$ mm)	P value
Group 1	—	DNFB	DNFB	11.3 ± 3.1 ‡	—
Group 2	+	DNFB	DNFB	5.0 ± 3.7	<0.01 §
Group 3	—	DNFB	Oxazolone	25.5 ± 4.2	—
Group 4	+	DNFB	Oxazolone	24.8 ± 5.3	NS¶

* Spleen cells from cell donors were transferred into recipient mice intravenously 5 days after sensitization of cell donors, and 1 h after transfer, recipient mice were sensitized with DNFB (Group 1 and 2) or oxazolone (Group 3 and 4).

† Hyperthermia treatments at 43°C for 45 min were given to cell donors (Group 2 and Group 4) on days 2 and 1 (date of sensitization was counted as day 0).

‡ Mean value $\pm$ SD is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

§ P value vs. Group by Student's *t*-test is shown.

¶ Not significant vs. Group 3 by Student's *t*-test.

TABLE 6. Transfer study of hyperthermia treatment on CS in mice—efferent phase study*

	Hyperthermia treatment† of cell donors	Sensitization of cell donors	Sensitization and elicitation of recipients	Ear swelling response ($\times 10^{-2}$ mm)	P value
Group 1	—	DNFB	DNFB	13.0 ± 2.2 ‡	—
Group 2	+	DNFB	DNFB	13.5 ± 5.7	NS§
Group 3	—	DNFB	Oxazolone	31.0 ± 5.3	—
Group 4	+	DNFB	Oxazolone	28.2 ± 4.6	NS¶

* Donor and recipient mice were sensitized on the same day, and 5 days after sensitization, spleen cells from cell donors were transferred intravenously into recipient mice. One h after transfer, recipient mice were elicited by DNFB (Group 1 and 2) or oxazolone (Group 3 and 4).

Hyperthermia treatments at 43°C for 45 min were given to cell donors (Group 2 and Group 4) on days -2 and -1 (date of sensitization was counted as day 0).

‡ Mean value $\pm$ SD is shown. Non-immunized mice were challenged similarly and their background ear swelling response was subtracted from those of immunized mice.

§ Not significant vs. Group 1 by Student's *t*-test.

¶ Not significant vs. Group 3 by Student's *t*-test.

that the suppressive effect is produced by both local and systemic mechanisms. Roszkowski *et al.*¹³ have reported that 7 consecutive days or more of whole body hyperthermia, performed for 2 h at a time, suppressed CS to oxazolone in mice. In our study, we have shown that only a single and local exposure to hyperthermia had a suppressive effect on CS both locally and systemically. We also determined that the suppressive effect of local hyperthermia was both temperature- and dose-dependent. Treatment at 42°C or higher had suppressive effects, however, not at 41°C. Therefore, it can be inferred that the suppressive effect is due to hyperthermia, but not to the stress the mice undergo during treatment. Furthermore, it was enhanced by repeated treatments and increasing the dose of a single treatment.

It has been reported that LCs are antigen presenting cells in the induction phase of CS and that CS is initiated by the recognition of an antigen on the surface of the LCs by T_{DTH} cells. The distribution of LCs as detected by the ATPase reaction is not uniform in the various portions of the body,¹⁴ and LC-poor skin, such as the tail skin, seems to lack certain essential functions relating to the immunoreactivity.¹⁵ In our experiments, a reversible decrease in the number of LCs after hyperthermia treatment was observed, and there is a clear positive correlation between the number of LCs and the extent of CS when sensitized at hyperthermia treated skin. From these results, it is suggested that the mouse skin loses the capacity to sustain CS following an exposure that depletes the LCs, but regains the ability with the regeneration of the LCs. This speculation is consistent with previous reports on the role of LCs in CS.¹⁶⁻¹⁸ It has also been reported that in *in vitro* studies, LCs were much more sensitive to hyperthermia treatment than other epidermal cells.¹⁹ The present study demonstrated that the suppressive effect of local hyperthermia on CS was transferred by antigen-specific suppressor cells generated by pre-exposure of the sensitizing site to hyperthermia with a resultant LC dysfunction. The suppressive effect was not transferred when the recipient mice were sensitized before transfer of spleen cells. These data show that the suppressor cells block the afferent limb of the CS reaction

but not the efferent limb. However, it is possible that other mechanisms are involved in the suppression in the afferent limb, because hyperthermia also suppressed CS systemically. The suppressive effect of hyperthermia on CS observed in our experiments seems to be similar to that of UVB (ultraviolet B) exposure¹⁶⁻¹⁸ or PUVA (psoralen and ultraviolet A) treatment.²⁰ However, the penetration of heat is much deeper than UV radiation, therefore, hyperthermia treatment may affect lymphatics and the regional lymph nodes as well as LCs in epidermis. This may be the reason why hyperthermia showed both local and systemic suppressive effects on CS. Further studies are necessary to elucidate the differences and the similarities of these treatments. If hyperthermia treatment is used on cancer patients whose immune system may be already suppressed, the inhibitory effect of hyperthermia on the host's immune system would be unfavourable to the patient. Thus, it is important to clarify the mechanism of the suppressive effect of hyperthermia treatment in order to treat cancer patients more effectively and safely.

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Triiodothyronine alters the cornification of cultured human keratinocytes

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SUMMARY

Scaly skin occurs in 80–90% of patients who are hypothyroid, the pathogenesis of which is unknown. Since thyroid hormone (T₃) affects growth and differentiation in other organs, we examined the effects of its absence on keratinocytes *in vitro*. Human neonatal foreskin keratinocytes were cultivated and second passage cells were switched to T₃-depleted (–T₃) medium at 50% confluence. Cells maintained in the –T₃ medium demonstrated increased (1.5 fold) levels of the cross-linking enzyme transglutaminase and increased (1.5 fold) formation of cornified envelopes, when compared to keratinocytes maintained in medium containing physiologic levels (2×10^{-9} M) of T₃. Additionally, in the –T₃ cultures, the level of the protease plasminogen activator (PA), an enzyme implicated in the process of shedding of cornified cells, was decreased 70–80% of that measured in +T₃ media.

Absence of T₃ from keratinocyte culture-medium increased both the level of the enzyme responsible for cross-linking cornified envelope precursors and the rate of envelope formation in cultured cells. The decreased levels of PA observed in the –T₃ cultures could result in decreased shedding of cornified cells. These alterations in the process of keratinocyte differentiation may explain the clinically observed scaliness associated with hypothyroidism in humans. The molecular mechanism by which T₃ alters keratinocyte cornification is not yet known.

Thyroid hormone affects the growth and differentiation of numerous organs in humans. Known target organs, such as the developing brain, are profoundly influenced by levels of thyroid hormone and deficiency in the neonatal period is evidenced by severe mental retardation. A deficiency of this hormone in later life also affects many other organs; the cardiovascular, gastrointestinal, nervous, and renal systems are most commonly affected. One of the clinical signs seen in hypothyroidism is a scaly skin. This suggests that the epidermis may be a specific target for thyroid hormone activity. A previous study examining the epidermis in hypothyroid adults demonstrated a decreased epidermal mass that was not necessarily correlated with a concomitant decrease in the rate of epidermal proliferation.¹ Although other authors have

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suggested that decreased levels of thyroid hormone are associated with decreased epidermal proliferation,^{2,3} a decrease in proliferative rates of the epidermis alone does not explain the formation of scale and the ichthyotic changes.

In the present study the role of triiodothyronine in the process of epidermal maturation was examined *in vitro*. During the course of normal epidermal differentiation, detergent-resistant cornified envelopes form and constitute the stratum corneum of the skin. These cells are normally shed (desquamated) from the skin surface by an, as yet, unclear mechanism. It has been suggested that a scaly skin may result from a number of epidermal abnormalities. It may be due to an increased rate of cornified envelope formation a decreased rate of desquamation, or due to an abnormal process of cornification.⁴ We examined the process of cornification in cultured human keratinocytes to determine how they were affected by the absence of thyroid hormone.

METHODS

Cell culture

Human epidermal keratinocyte cultures were prepared from neonatal foreskins using a modification of the method of Rheinwald and Green⁵ as previously reported.⁶ The cells were subcultured into plates pre-seeded with mitomycin C-treated 3T3 cells as a feeder layer. All experiments were done with either second or third passage cultures. When cultures attained 50% confluence the dishes were washed twice with phosphate-buffered saline (PBS) and experimental medium added. Thyroid hormone-depleted ($-T_3$) medium consisted of Dulbecco's modified Eagle's medium (DMEM) with 10% serum from a thyroidectomized calf (TXS) (Rockland Farms, PA, U.S.A.), hydrocortisone (0.4 μ g/ml), epidermal growth hormone (EGF) (1 ng/ml), and cholera toxin (10 ng/ml). L-triiodothyronine (Sigma, St. Louis, MO, U.S.A.) at 2×10^{-9} M, added to the thyroid-deficient medium constituted the thyroid hormone-repleted ($+T_3$) medium. Radioimmunoassay of the growth medium (DMEM + 10% TXS), as well as the TXS, revealed undetectable levels of both T_3 and T_4 . The T_3 -supplemented medium had undetectable levels of T_4 and a T_3 level of 1.2–1.4 ng/ml (2×10^{-9} M). These levels are comparable to the level of total T_3 in human serum.⁷ Cultures were fluid changed twice weekly and sprayed with 0.02% EDTA at 70% confluence to free them of any remaining 3T3 feeder cells or contaminating fibroblasts.

Enumeration of cell number, cornified envelopes

To calculate the fraction of cells containing cornified envelopes, 7- or 14-day-old cultures were vigorously washed twice with buffer to remove any unattached envelopes prior to the collection period. The cultures were subsequently incubated for 48 h. The cells shed into the medium over this period and those dislodged by three rigorous washes of the culture surface were collected by low-speed centrifugation. The cells adherent to the culture dish were removed from the plate by standard treatment with 0.25% trypsin–0.02 mmol/l EDTA, and collected by centrifugation.

Scoring was done using a modification of Green's published procedure.⁸ The cell pellet of either the shed or the adherent cells was washed and resuspended by vigorous pipetting in 1 ml of buffer. To determine the total cell number, an aliquot of this cell suspension was counted in a Coulter counter and checked by manual haemocytometer counting. The remaining cells were collected by low-speed centrifugation and resuspended in 20 mmol/l Tris-HCl, pH 7.7 containing 1% β -mercaptoethanol (BME) and 1% SDS. After incubation for 10 min at room temperature, cells were counted in a haemocytometer to determine the number of detergent and BME-resistant envelopes which appear as clear cell ghosts.

Determination of cell-associated plasminogen activator (PA) levels

Cells to be assayed for PA were washed twice with cold phosphate-buffered saline, scraped off the plate with a rubber policeman, and collected by centrifugation. The cell pellet was disrupted by sonification for 15 2-s bursts (Sonifier Cell Disrupter, Heat Systems-Ultrasonics, Plainview, NY, U.S.A.) in ice-cold 0.1 mol/l Tris-HCl, pH 8.1, containing 0.5% Triton X-100. The cell extract was then centrifuged for 20 min at 20,000 *g* at 4°C and aliquots of the supernatant frozen for PA assay and protein determination.

The (¹²⁵I)-fibrin-coated multiwell assay was used to determine PA levels, as previously described.⁹ Briefly, 2 or 5 µg of cell lysate protein and purified FCS-plasminogen were added to a (¹²⁵I)-fibrin-coated microwell. The dishes were incubated at 37°C and an aliquot of the supernatant fluid assayed for solubilized (¹²⁵I)-fibrin degradation products at 1, 2, and 4 h. All assays were performed under conditions of substrate (plasminogen) excess, and only values in the linear range of the assay with respect to both time and sample concentration were used. PA was determined as the mean value of duplicate assays on each of two samples and expressed as milliunits (Ploug) of Urokinase Reference Standard (Leo Pharmaceutical Products, Ballerup, Denmark) per µg cell lysate protein. All data are expressed here as the percent of values obtained in T3-supplemented cultures, normalized to 100%. Control assays of cell extracts in the absence of plasminogen revealed no plasminogen-independent fibrinolysis.

Determination of cellular transglutaminase levels

Transglutaminase activity was measured in keratinocyte lysates as described by Lichte and co-workers.¹⁰ Twice-washed culture dishes were placed on a bed of dry ice and scraped into buffer containing 0.5 ml of 20 mmol/l phosphate buffer, pH 7.2, 10 mmol/l dithiothreitol, 0.5 mmol/l EDTA. The cell slurries were maintained on ice and sonicated 15 × 2 sec, centrifuged at 10,000 *g* in a refrigerated Beckman Airfuge, and the supernatant used in enzyme assay. The assay mixture contained 50 µl of cellular supernatant and 150 µl of freshly prepared sodium borate buffer 50 mmol/l pH 9.5, containing 10 µl of 100 mmol/l CaCl₂, 10 µl of 10 mmol/l EDTA, 20 µl of casein (20 mg/ml, Sigma, St. Louis, MO, U.S.A.), 10 µl of [1,4-¹⁴C]-putrescine, 118 Ci/mmol (Amersham Corp., Arlington Heights, IL, U.S.A.), and 80 µl of distilled H₂O. Samples and blanks were incubated at 37°C for 30 min, and duplicate aliquots spotted on Whatmann 3 mm paper. Macromolecules were precipitated with 10% TCA and the filters placed into scintillant (Aquasol-2, Dupont New England Nuclear, Boston, MA U.S.A.) and radioactivity measured in a scintillation counter. Protein was measured using the method of Higuchi and Yoshida.¹¹ Results of duplicate determinations on each of two samples were expressed as CPM putrescine incorporated/µg cell lysate protein, and are represented here as the percentage of values obtained in T3-supplemented cultures, which have been normalized to 100%.

Other determinations

T3 and T4 in experimental culture media were quantitated by radioimmunoassay at the University of California, Davis Medical Center (Centria Total T3 and T4 Radioimmunoassay Test Sets, Ventrex Laboratories, Inc., Portland, ME, U.S.A. Lower limits of detection are: T3: 0.15 ng/ml, T4: 5.0 ng/ml). For statistical analysis, paired Student's *t*-test, two tailed, with significance assigned at *P* ≤ 0.05 was utilized.

RESULTS

Effect of T₃ absence on rates of keratinocyte shedding and cornified envelope formation

In the keratinocyte culture system, as cells differentiate, they slough off into the medium, by a process thought to be analogous to *in vivo* desquamation. The number of cells attached to the culture dish or shed into the medium were determined in the absence of T₃ and in control cultures that were supplemented with T₃. There was a significant decrease (44% control) in the rate of cell shedding as a percentage of the total cell population in the -T₃ cultures (Fig. 1). Decreases (80%) in number of cells both adherent to and shed from the culture dish, though not statistically significant, were noted in the -T₃ cultures, as well.

Terminal differentiation of keratinocytes was also examined by quantitating the number of cornified envelopes. After 14 days of cultivation in either T₃-depleted or T₃-supplemented medium, the number of detergent and BME-resistant envelopes was enumerated in both the adherent and shed cell populations. The most significant finding was the increase in cornified envelopes in the -T₃ cultures: a 1.3 fold increase ($P=0.05$) in the number of cornified envelopes present in both the adherent and shed keratinocyte populations. The percentage of envelopes present in the -T₃ cultures (total cell number) was 1.5 fold increased.

Effect of T₃ on transglutaminase levels

Levels of total keratinocyte transglutaminase, the enzyme necessary for crosslinking of envelope precursors, were elevated in the -T₃ cultures. Figure 2(a) reveals that after both 7 and 14 days

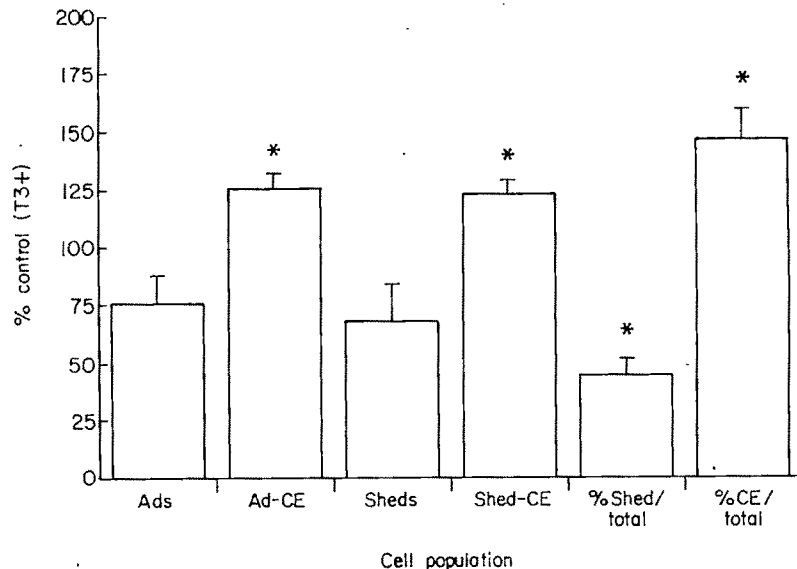


FIGURE 1. Effect of T₃ absence on cornified envelope formation and keratinocyte shedding. Human keratinocytes were cultivated in T₃-depleted or T₃-supplemented medium for 14 days. The numbers of cells adherent to culture dish (Ads) and shed from culture dish into the medium (Shed) were determined, and the percentage of BME and detergent resistant cornified envelopes in each population of cells was determined as outlined in Methods (Ad-CE) (Shed-CE). Values represent mean $\pm$ SEM ($n=3-4$) and are expressed as percent of values obtained in control (T₃-supplemented cultures). Asterisk marks values statistically different from control, $P \leq 0.05$ by Student's *t*-test.

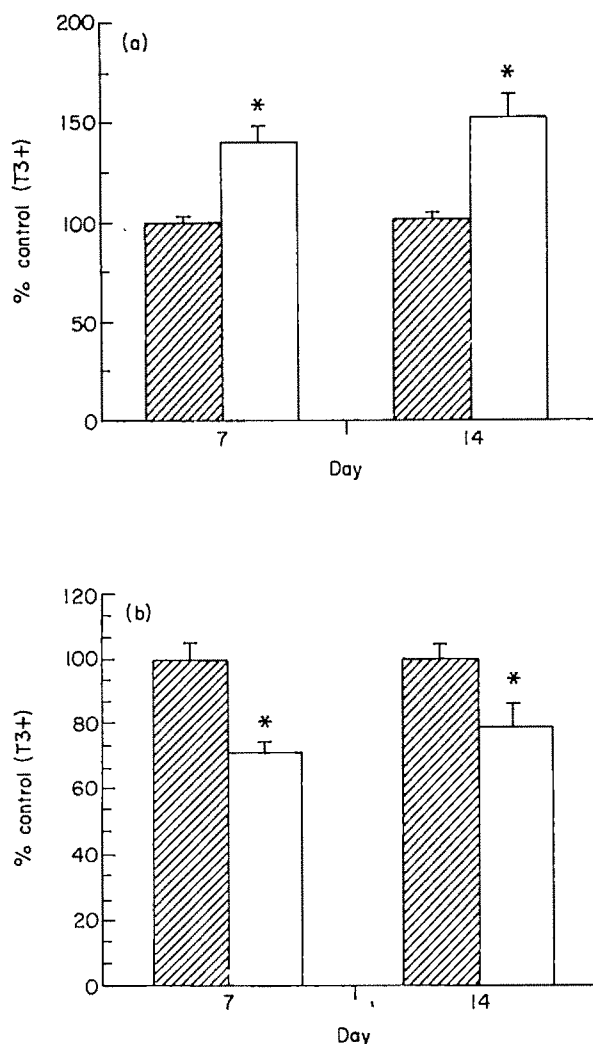


FIGURE 2. Effect of T₃ absence on keratinocyte transglutaminase (a) and plasminogen activator (b) levels. ▨ T₃-supplemented cultures, □ T₃-depleted cultures. Values = mean $\pm$ SEM ($n=4-6$), expressed as percentage of values obtained in control (T₃-supplemented), which have been normalized to 100%. * = values statistically different than control, $P \leq 0.05$.

of cultivation, significant increases in the cellular levels of this enzyme could be detected in the $-T_3$ cultures.

Effect of T₃ on plasminogen activator levels

Since several investigators have suggested that the serine protease plasminogen activator (PA) may facilitate cornified envelope detachment and shedding^{9,12} and the rate of shedding was decreased in the $-T_3$ cultures, levels of this enzyme were analysed in the $+T_3$ and $-T_3$ cultures. Figure 2(b) demonstrates a significant decrease in the cell associated levels of PA in cultures maintained for 7 or 14 days in $-T_3$ medium.

TABLE 1. Reversal of abnormalities in T₃-depleted cultures by addition of thyroid hormone

	Control (+T ₃)	T ₃ -depleted (-T ₃)	Switched back to T ₃ (-T ₃ /+T ₃)
Cell number			
Adherent cells	4.80 × 10 ⁵ (± 0.1)	1.86 × 10 ⁵ * (± 0.4)	3.89 × 10 ⁵ (± 0.8)
Shed cells	7.80 × 10 ⁴ (± 0.4)	2.70 × 10 ⁴ * (± 1.1)	3.17 × 10 ⁴ (± 1.1)
% Cornified envelopes			
Adherent cells	9.1 (± 0.8)	12.8 (± 3.3)	7.9† (± 3.6)
Shed cells	22.4 (± 7.3)	71.5 (± 23.8)	80.5 (± 19.4)
Total cells	10.9 (± 0.3)	21.1 (± 6.8)	13.1 (± 4.4)
Transglutaminase (cpm/μg protein)	419.7 (± 29.6)	774.7* (± 82.0)	587.4† (± 29.7)

Values represent means of two experiments ± SEM. * Values that differ significantly ($P < 0.05$) from control (+T₃) † Values that significantly differ ($P < 0.05$) from T₃-depleted (-T₃) cultures.

Reversal of abnormalities in -T₃ cultures by addition of thyroid hormone

To ascertain that the observed biochemical and cellular changes noted in the -T₃ cultures could be reversed by the addition of T₃, some cultures which had been maintained in -T₃ medium for 7 days were switched to +T₃ medium and incubated for an additional 7 days. Cultures maintained in -T₃ medium for 14 days and those switched to +T₃ medium after 7 days were compared to control +T₃ cultures which had been maintained for 14 days in +T₃ medium. Addition of thyroid hormone to the previously T₃-deficient cultures resulted in changes in the levels of transglutaminase, cell numbers, and percentages of cornified envelopes to more closely resemble the values in the control, +T₃ cultures (Table 1). Thus, the alterations in cornification induced in the thyroid-deficient cultures could be reversed by the addition of T₃ to the culture medium.

DISCUSSION

The factors which normally control differentiation in human epidermal keratinocytes are not fully understood. A number of physiologic substances such as retinoids, EGF,¹³ vitamin D,¹⁴ and calcium¹⁵ have been suggested as possible putative regulators of differentiation *in vivo*. Since abnormal keratinocyte differentiation, as manifest by epidermal scaling, is seen in 80–90% of patients with thyroid hormone deficiency, it seems reasonable to suggest that thyroid hormone may be another physiologic regulator of keratinocyte function. The studies presented here do indeed demonstrate that T₃ can affect keratinocyte differentiation. Absence of the hormone in the keratinocyte culture-medium induces increases in the levels of transglutaminase, the enzyme responsible for cross-linking precursors of the keratinocyte cornified envelope, as

well as concomitant increases in the numbers of cornified envelopes produced by the cultures. Additionally, levels of the enzyme plasminogen activator are reduced in the thyroid-deficient cultures. This enzyme has been implicated in the process of shedding or desquamation of cornified envelopes from the uppermost layer of the epidermis.^{9,12} Therefore, the decreased levels of this enzyme in thyroid hormone-deprived cultures may explain the finding of increased retention of cornified envelopes in the adherent cell layer in the *in vitro* system. *In vivo*, this retention of cornified envelopes may be ultimately manifested as scaliness.

Epidermal differentiation represents a co-ordinated programme of a number of changes including, among others, the increased expression of high molecular weight keratins and their intermolecular disulphide cross-linking, synthesis of the cornified envelope precursor, involucrin, formation of the cornified envelope, accumulation and processing of a histidine-rich protein, filaggrin and sloughing of differentiated cells.¹⁶ Normally, these events occur in a precisely timed sequence in the epidermis. Although a number of putative regulators of differentiation (noted above) have been identified, individual factors induce some, but not all, of the markers of terminal differentiation. For example, vitamin D induces cornified envelope formation and sloughing in cultured murine keratinocytes without altering the pattern of keratin synthesis.¹⁴ Hence, the different aspects of keratinocyte differentiation may be under the control of different factors. This appears to be the case for the role of thyroid hormone, as well. Its presence decreases cornification but does not appear to alter keratin synthesis. A similar alteration in the cornification of bronchial epidermoid carcinoma cells has been noted by Miyazaki and colleagues.¹⁷ Levels of T₃ of 10⁻¹⁰ mol/l or higher, inhibited cornified envelope formation in these cells as well.

It is of interest to note that thyroxine promotes shedding, or moulting, of cornified cells or feathers in birds, reptiles and amphibians.¹⁸ Thyroidectomy results in thickened skin in reptiles as a result of decreased epithelial shedding. Our results demonstrating decreased shedding of cells in the thyroid deficient cultures are consistent with these observations. One possible explanation for the decreased shedding may derive from the observation that the thyroid-deficient keratinocytes also express decreased levels of the protease plasminogen activator, which has been implicated in the processes of keratinocyte shedding.

The molecular mechanisms by which T₃ affects cornification and cellular shedding are, at present, unknown. We have previously demonstrated that thyroid-deficient keratinocyte cultures manifest decreased levels of neutral lipid, and specifically, cholesterol and cholesterol-esters.⁶ Although abnormalities in epidermal cholesterol synthesis have been associated with abnormal structure and function of the stratum corneum,¹⁹ further studies are necessary to determine whether epidermal lipid abnormalities, induced by thyroid deficiency, are involved in the molecular control mechanism for cornification or, rather, are co-observed phenomena. Nevertheless, our data demonstrate that thyroid hormone, and its deficiency can distinctly affect the pattern of human keratinocyte cornification *in vitro*. Although the applicability of these findings to the *in vivo* situation cannot be made without further study, it seems likely that the thyroid hormone does regulate keratinocyte function *in vivo* because of the epithelial abnormalities that accompany 80–90% of patients with clinical hypothyroidism. Hence, this study provides some insight as to the mechanism by which hypothyroidism clinically effects the skin.

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An assessment of the value of Ag NOR staining in the identification of dysplastic and other borderline melanocytic naevi

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SUMMARY

One hundred and six melanocytic lesions were studied to determine the value of the so called Ag NOR technique in differentiating dysplastic naevi, Spitz naevi and spindle cell naevi of Reed from malignant melanoma and from 'banal' compound or intradermal naevi. In 79 cases Ag NOR counts were possible. The banal naevi (36) had a mean count of 1.54, (SD 0.3), and the unequivocally malignant superficial spreading melanomas (13), lentigo maligna melanomas (4) and secondary melanomas (4) had a mean count overall of 3.9 (SD 1.59). For dysplastic naevi (16) the mean Ag NOR count was 1.63 (SD 0.36) and for Spitz and spindle cell naevi (*in toto* 6) the figure was 1.72 (SD 0.55). The difference in Ag NOR counts between all types of naevi and all types of melanoma was highly statistically significant, but there was no difference between banal naevi and dysplastic, Spitz, and spindle cell naevi. Correlation of Ag NOR counts between three independent observers was good. This technique may, therefore, be a useful adjunct in separating true melanoma from borderline melanocytic naevi.

Accurate histological diagnosis of cutaneous malignant melanoma is an essential, and at times, difficult task for the histopathologist. In the past 5 years, the use of monoclonal antibodies such as NKI C3¹ and antibody to S100 protein² in the immunoperoxidase technique have proved valuable in identifying an indeterminate tumour mass to be of melanocytic origin. These antibodies do not, however, separate benign from malignant melanocytic lesions, and although a wide range of antimelanoma monoclonal antibodies is now available, some of which identify differentiation and tumour progression markers, the majority of these antibodies react only on frozen tissue. They are, therefore, impractical for routine diagnostic use or retrospective analyses. An alternative approach to the differentiation between malignant melanoma and benign melanocytic naevi is the use of the Ag NOR technique. This technique has been developed and intensively studied by Crocker and his colleagues.^{3,4,5} Nucleolar organizer

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regions (NORs) are loops of ribosomal DNA which are seen in nuclei and ultimately direct both ribosome and protein formation.⁶ In man NORs are seen on the five acrocentric chromosomes 13-15, 21, 22. The Ag NOR technique described below identifies argyrophilic NOR-associated proteins and is highly specific. Early work on Ag NORs was mainly confined to cytogenetic laboratories and used in the evaluation of trisomies, but the valuable contribution of Ploton⁷ and colleagues in modifying the technique for routine use in paraffin wax-embedded tissue at room temperature has made the Ag NOR technique practical for routine pathological use.

Initially Crocker's group reported a clear correlation between numbers of Ag NORs and high and low grade on Hodgkin's lymphoma.³ High grade tumours had large numbers of Ag NORs and low grade tumours, only one or two with virtually no overlap. More recently Crocker and Skilbeck have reported results in a series of 50 melanocytic lesions comprising 18 malignant and 32 benign naevi of varying types.⁴ No benign melanocytic naevus had Ag NOR counts of greater than two, and no malignant lesion had Ag NOR counts of less than four. Thus, again, there was an unequivocal separation between benign and malignant lesions. In this study, however, no lesions were studied with either borderline or equivocal pathology, and no group of so called dysplastic naevi was included. This is an area in which aids to current conventional pathological methods would be of value.

We have, therefore, carried out a study of Ag NOR numbers in a range of melanocytic lesions. Initially we studied unequivocally benign and malignant lesions to establish our own laboratory's normal range, and, thereafter, proceeded to study dysplastic naevi. We also counted Ag NORs in a small number of Spitz naevi, and spindle cell naevi of Reed.

Additional aspects of our study were an assessment of interobserver variation in Ag NOR counts, and an assessment of the correlation if any between Ag NOR counts and Breslow thickness in the malignant melanomas.

METHODS

From our files of routinely formalin fixed, paraffin wax-embedded material, 3 μ m sections were cut from 20 superficial spreading melanomas, six lentigo maligna melanomas, three nodular melanomas, four secondary melanomas, 46 banal compound and intradermal naevi, 21 dysplastic naevi, three Spitz naevi and three spindle cell naevi of Reed. Sections were taken to water via xylene and graded ethanols. The Ag NOR solution was prepared by dissolving gelatine in 1 ml/dl of aqueous formic acid at a concentration of 2 g/dl. This solution was then mixed (1 to 2 volumes) with 50 g/dl of aqueous silver nitrate solution to give the final working solution. This was poured over the tissue sections and left for 30 min at room temperature in the dark room. Preliminary studies had established this incubation period as optimal for our observations. Silver colloid was then washed off with distilled deionized water, sections dehydrated through graded ethanols to xylene and then mounted in mounting media.

Three independent observers counted 100 naevus or melanoma cells under a $\times 40$ dry objective or $\times 100$ oil immersion. In individual nuclei both isolated Ag NORs and clusters were seen. Individual Ag NORs were found relatively easy to count but clusters were at times difficult to differentiate from melanin pigment and very difficult to count accurately. Preliminary studies with melanin bleach, in cases with heavy melanin pigment present, revealed no Ag NORs after the bleaching procedure. In cases where there was heavy melanin pigment deposition Ag NOR counts were not, therefore, attempted because of doubts about accuracy.

Results were subjected to statistical analysis using the Mann-Whitney *U*-test for non parametric data.

RESULTS

We found no difference between Ag NOR counts obtained with the $\times 40$ and $\times 100$ oil objectives, and a good correlation between Ag NOR counts obtained by the three observers. Initially the individual with least dermatopathological experience produced slightly higher counts than the other two individuals, but this was quickly overcome, and was not, thereafter, a problem, provided the heavily melanized sections were excluded.

The means of the Ag NOR counts in each group together with the standard deviations are shown in Table 1. It will be seen that in the case of the banal melanocytic naevi (compound and intradermal melanocytic naevi) Ag NOR counts are extremely low. In contrast, high Ag NOR counts are seen in the malignant melanocytic lesions. When these lesions are divided into lentigo maligna melanoma, superficial spreading melanoma, and secondary melanoma it will be seen that all types of melanoma show high Ag NOR counts with no statistically significant difference between them. Thus, this first part of the study showed that we obtained a clear statistically significant difference in Ag NOR counts between unequivocally benign and unequivocally malignant lesions.

The dysplastic naevi and the Spitz naevi and spindle naevi of Reed all showed low Ag NOR counts in the same range as the banal naevi with no significant difference between mean counts for the banal group of compound and intradermal naevi and the more difficult naevi. In the dysplastic naevi however it was relatively common to see the majority of cells with only one Ag NOR visible, but occasional small groups of cells with two or three Ag NORs present. These clusters were situated randomly, and were not always adjacent to the dermo-epidermal junction.

In this series we found that in 27 (25%) cases it was not possible to carry out counts on an adequate number of cells to give a meaningful result. This was usually due to melanin pigment masking the Ag NORs. Representative areas of dysplastic naevi, melanomas and a slide considered uncountable because of pigment are illustrated in Figures 1-3.

In this series no primary melanoma had a Breslow thickness greater than 2.85 mm. Within

TABLE 1. Numbers of lesions studied, lesions countable, Ag NORs and standard deviations

Lesion	No. studied	Countable	No. AgNORs	SD	
Naevi					
Banal compound and intradermal naevi	46	36	1.54	0.3	
Dysplastic naevi	21	16	1.63	0.36	All naevi 1.65 (0.4)*
Spitz naevi and spindle cell naevi of Reed	6	6	1.72	0.55	
Melanomas					
Superficial spreading melanoma	20	13	4.1	1.63	
Lentigo maligna melanoma	6	4	3.03	1.35	All melanomas 3.9 (1.59)*
Nodular melanoma	3	0			
Secondary melanoma	4	4	4.18	1.76	

* Statistically significant differences. $P < 0.0001$.

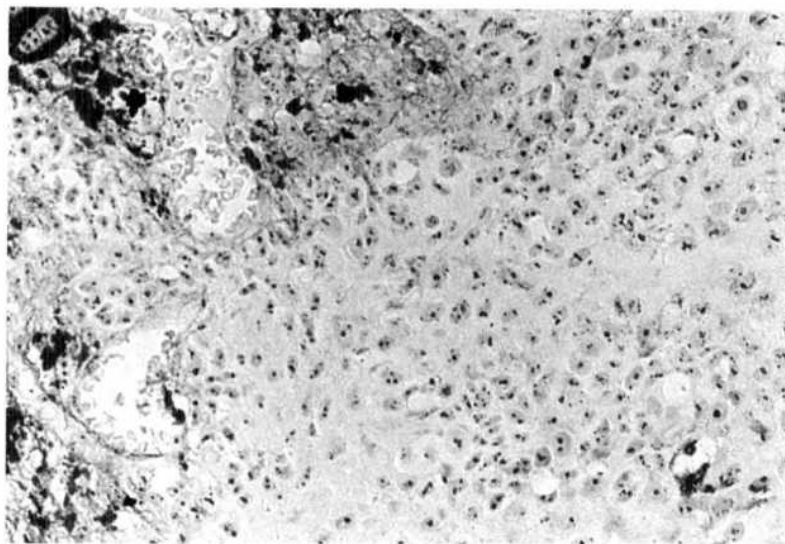


FIGURE 1. Primary melanoma showing large numbers of Ag NORs in the majority of cells ($\times 40$).

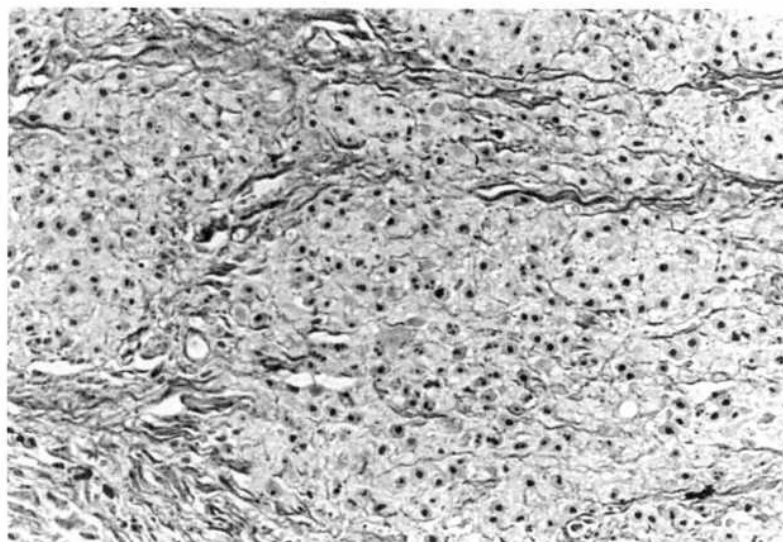


FIGURE 2. Dysplastic naevus. The majority of cells have only one large Ag NOR, but a few have two or three ($\times 40$).

this range, there was no clear correlation between tumour thickness and Ag NOR counts, and there was no difference in Ag NOR counts between primary and secondary melanomas.

DISCUSSION

We are aware of two other papers in print studying Ag NORs in melanocytic lesions. Crocker and Skilbeck⁴ report very similar results for their smaller series of banal naevi, but higher Ag

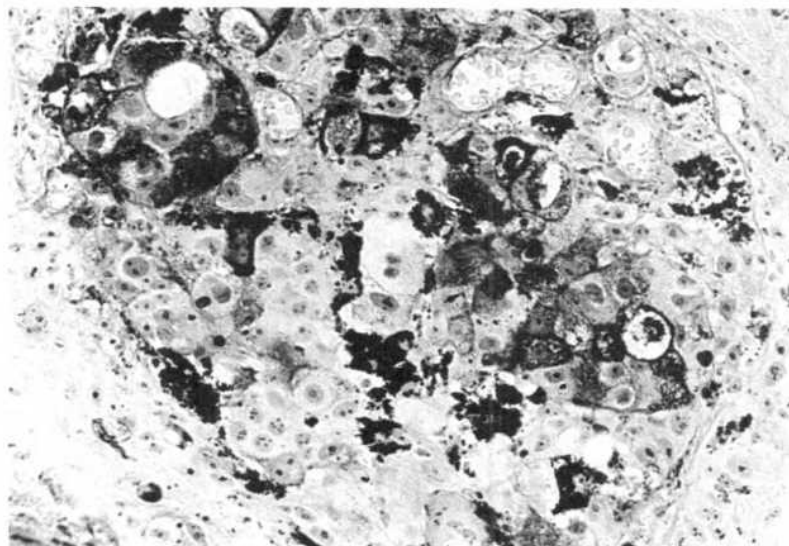


FIGURE 3. Example of nodular melanoma considered unsuitable because of pigment ($\times 40$).

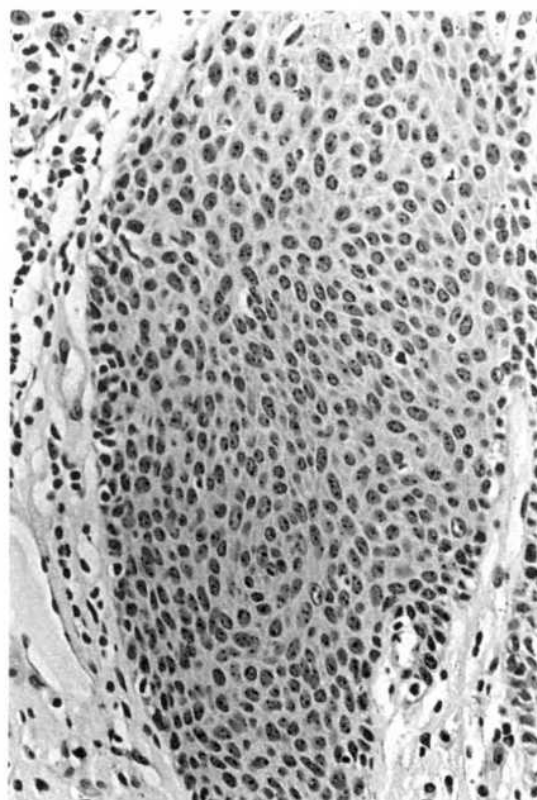


FIGURE 4. Pseudoepitheliomatous hyperplasia overlying Spitz naevus. Note that many keratinocytes have two or three Ag NORs ($\times 40$).

NOR counts in malignant melanocytic lesions. Direct comparison is not possible between their results and this present report as their division of malignant lesions is into lentigo maligna, superficial spreading melanoma, and 'melanocarcinoma'. They did not, however, include any dysplastic naevi or borderline lesions in their study and this is clearly the area in which aids to histological diagnosis are most needed.

A recent publication by Fallowfield Dodson and Cook⁸ reports on a population similar to that studied by ourselves. They also found low Ag NOR counts for both banal and dysplastic naevi with mean counts of 1.16 and 1.18 respectively, and high counts for melanoma with a mean of 7.64. In this paper it is also stated that Ag NOR counts above one are not infrequently seen in the epidermis surrounding both benign and malignant melanocytic lesions. We also noted this phenomenon, and Figure 4 illustrates an area of pseudoepitheliomatous hyperplasia overlying a Spitz naevus. If the oil immersion objective is chosen for counting, it is clearly important to carefully identify the cell type prior to counting 100 cells.

After the initial clearcut results reported in both lymphomas and melanocytic lesions, a more recent paper on breast malignancies reports no correlation between mitotic rates and Ag NOR counts, and suggest that the relationship to ploidy may be more relevant.⁵ Mitotic counts show considerable inter-observer variation and further work is needed in this area. A further recent publication has suggested that what are currently counted as Ag NORs may in fact be nucleoli.⁹ This also requires further study.

Walker¹⁰ has commented on the need for correlation of variation with fixative, section thickness and observer. In this present study, the fixative was in all cases neutral buffered formalin, the section thickness was 3 μ m, and the inter-observer error was low after initial discussions. No other study has given a figure for the sections discarded as uncountable because of melanin pigment or a lack of an adequate number of cells. In our series this was relatively high at 25%, and we cannot agree with the statement by Crocker and Skilbeck⁴ that melanin was never a problem.

In summary, we suggest that the Ag NOR technique may be of value in differentiating the difficult or borderline naevus from the frank malignant melanoma.

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The normal range in diagnostic phototesting

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SUMMARY

The minimal erythema doses (MED) to UVB and UVA radiation were measured in 254 normal subjects. Bivariate correlation analysis applied to the data showed a strong positive correlation between the UVB and UVA MEDs. By calculating the probability that a given combination of UVB and UVA MEDs is likely to occur in normal subjects, it was shown that, in some instances, both the UVB MED and UVA MED observed in a given subject may be within their respective normal ranges, but that the particular combination is more in keeping with abnormal photosensitivity.

The object of diagnostic phototesting is to determine whether or not the response of the skin to a given ultraviolet radiation (UVR) spectrum is abnormal. The radiation is usually administered to skin of normal appearance in a geometric series of increasing exposure doses. A geometric series implies a constant ratio between successive doses, unlike an arithmetic series which involves increasing each dose by a constant amount, and is normally more economical than an arithmetic series in covering a given dose range, since fewer exposures are required. This results in less skin area exposed and a shorter irradiation time. The response of the skin is generally assessed visually in terms of the delayed erythema present at 24 h after irradiation. It is conventional practice to determine the exposure dose of UVR that results in just perceptible reddening of the skin, and to use this 'minimal erythema dose' (MED) as an index of cutaneous photosensitivity.^{1,2}

The UVR sources for phototesting include mercury or xenon arc lamps used in conjunction with either optical filters or a monochromator to isolate narrow spectral wavebands. The choice of spectral regions in which a patient may be irradiated depends on the diagnosis, but it would be unusual if a patient was not irradiated with sunburn wavelengths around 300 nm (UVB) and with longer wavelength UVR in the region 320–400 nm (UVA).¹

Once the MEDs to, e.g. UVB and UVA radiation have been established, the investigator compares these values with the MEDs expected in a 'normal' population in order to decide whether or not the patient is exhibiting abnormal photosensitivity. The 'normal ranges' (95% confidence interval) for UVB and UVA irradiation in British subjects encompass a factor of four

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in MED.^{3,4} Consequently, a subject may have a UVB MED at the high end of the normal range, with a UVA MED at the low end of the normal range, and be classified as normal. Intuitively we might expect the UVB and UVA MEDs in normal skin to be correlated, and in an example such as this, it might be imagined that the subject was exhibiting signs of abnormal sensitivity to UVA radiation. The purpose of the present analysis is to examine whether any correlation exists between UVB and UVA MEDs in normal subjects and, if so, to use this information to increase the likelihood of demonstrating abnormal photosensitivity.

METHOD

The data set consisted of 254 subjects phototested in the Medical Physics Department at Dryburn Hospital over a 7-year period using an irradiation monochromator.⁴ The subjects comprised normal volunteers together with patients who, although originally referred for monochromator testing because of suspected photosensitivity, were subsequently classed as 'normal' as far as erythema response was concerned, on the basis of clinical assessment and the test results. Phototesting was carried out on skin of normal appearance on the upper back, as this area exhibits a higher sensitivity⁵ and a more uniform response⁶ to ultraviolet radiation than other sites. Although our phototesting protocol involved irradiating subjects in up to eight different spectral intervals, all patients were irradiated at a central wavelength of 300 nm with a full bandwidth at half maximum intensity of 5 nm (termed the 'UVB waveband'), and at 350 nm with a bandwidth of 30 nm (termed the 'UVA waveband'). A colour glass filter (Schott WG335) is inserted into the radiation beam for UVA irradiation in order to eliminate stray UVB and UVC radiation. For each spectral region, radiant exposures were increased geometrically by a ratio of $\sqrt{2}$, thus doubling alternate exposures.

The number of subjects having a given UVB and UVA MED is shown in Table 1. This data set, in which a given entry in the Table refers to two variables (the UVB and UVA MEDs), comprises a bivariate distribution. Mackenzie³ has shown that the MEDs in a given spectral waveband from a normal population are distributed 'lognormally'. This means that if the UVB and UVA MEDs are transformed logarithmically we can apply correlation analysis appropriate for bivariate normal distributions.⁷ The mathematical details of this statistical analysis are summarized in the Appendix.

TABLE 1. Number of normal subjects with a given UVB (300 $\pm$ 5 nm) and UVA (350 $\pm$ 30 nm) minimal erythema dose (MED)

UVA MED (J/cm ²)	UVB MED (mJ/cm ²)							
	10	14	20	28	40	56	80	112
10	0	0	0	0	0	0	0	0
14	0	2	5	3	1	0	0	0
20	0	1	24	31	20	11	0	0
28	0	1	12	28	33	29	0	0
40	0	0	5	14	15	10	5	1
56	0	0	0	0	0	1	0	2
80	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0

RESULTS

Correlation analysis applied to the data in Table 1 indicated a positive correlation ($r=0.56$; $P<0.0001$) between the UVA MED and the UVB MED. The median MEDs and 'normal ranges' (95% confidence interval) for UVB and UVA radiation are summarized in Table 2. Table 3 indicates the probability (calculated from Equation 7 in the Appendix) that a normal subject from our population exhibits a given UVA/UVB MED combination.

TABLE 2. Results of bivariate regression analysis on 254 normal subjects

Radiation	Minimal erythema dose	
	Median	95% confidence interval
UVB	34 mJ/cm ²	14-84 mJ/cm ²
UVA	26 J/cm ²	14-52 J/cm ²

TABLE 3. The probability that a subject exhibits normal photosensitivity for a given UVB (300±5 nm) and UVA (350±30 nm) minimal erythema dose (MED)

UVA MED (J/cm ²)	UVB MED (mJ/cm ²)							
	10	14	20	28	40	56	80	112
10	**	*	*	*	**	***	***	***
14	*	N	N	N	N	**	***	***
20	*	N	N	N	N	N	*	**
28	**	*	N	N	N	N	N	*
40	***	**	*	N	N	N	N	*
56	***	***	**	*	N	N	N	*
80	***	***	***	***	**	**	**	**
112	***	***	***	***	***	***	***	***

Symbol *P*-value.

N = $P > 0.05$.

* = $0.01 < P \leq 0.05$.

** = $0.001 < P \leq 0.01$.

*** = $P \leq 0.001$.

DISCUSSION

The median UVB and UVA MEDs given in Table 2 are very similar to those published by us 5 years ago⁴ for normal subjects. We can infer from this that it is unlikely that there has been a systematic change with time in ultraviolet radiation dosimetry associated with our phototesting procedures, or in the sunburn susceptibility of our local population.

The 95% confidence intervals in Table 2 encompass a range of six in UVB MED but only four in UVA MED. The reason for this is probably related to the ultraviolet optics of skin. A given

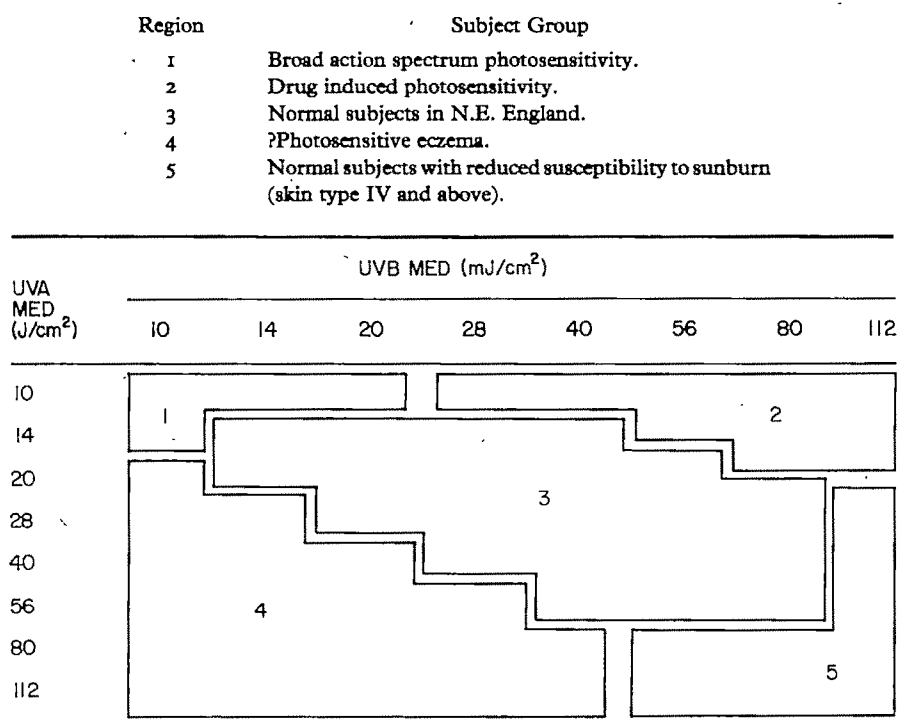
change in both epidermal thickness⁸ and melanin pigmentation⁹ will have a more pronounced effect on the flux of UVB reaching critical targets in the skin, and hence the MED, than on the flux of UVA radiation.

The results of bivariate regression analysis supported the expectation that, in a given subject, the UVB and UVA MEDs are correlated. The correlation coefficient r (Equation 5 in Appendix) had a numerical value of $+0.56$. The positive correlation indicates that the UVA MED increases with the UVB MED. The square of the correlation coefficient is equal to 0.31 . The interpretation⁷ of this is that about 30% of the variance in the UVB MEDs is explained by variability in the UVA MEDs.

In Table 3 the symbol 'N' is used to indicate those combinations of UVA and UVB MED that we would expect to be observed in 95% of our 'normal' subjects. This is not to say that subjects who are observed to have a particular UVA and UVB MED indicated by one or more stars (*) are necessarily abnormal. The subjects we studied were drawn from those living in North East England and were sun-reactive skin types¹⁰ of I, II, III, IV. Mostly they were II or III, rarely I or IV. The 'normal ranges' illustrated by Table 3 are, therefore, a biased sample of the total population; indeed, they may even be a biased sample of U.K. residents. This means that we would be very unlikely ($P < 0.001$) to phototest a subject whose UVA and UVB MEDs were 112 J/cm^2 and 112 mJ/cm^2 , respectively. It does not mean that a subject exhibiting this combination of MEDs is pathologically abnormal, just that he is likely to be skin type V, and not many people with this complexion live in the our catchment area.

We have indicated this group of subjects, who are essentially normal, but who are very

TABLE 4. Approximate classification of subjects according to their UVB ($300 \pm 5 \text{ nm}$) and UVA ($350 \pm 30 \text{ nm}$) minimal erythema dose (MED)



unlikely to present for phototesting, by region 5 in Table 4. Region 3 in this Table indicates those subjects in our local population whom we would regard as having 'normal' UVA and UVB MEDs. Patients with abnormal broad action spectrum photosensitivity, such as seen in the idiopathic eczematous photosensitivity known as chronic actinic dermatitis,¹¹ fall into region 1.

Region 2 comprises those subjects who have a normal UVB MED but a UVA MED which is uncharacteristically low. We find that patients with a drug-induced photosensitivity often fall into this category. Within region 2 there exist both UVB and UVA MEDs that lie within their respective 'normal ranges'. For example, a subject whose UVB and UVA MEDs were 56 mJ/cm² and 14 J/cm² would, on the basis of Table 2, be regarded as normal. Since the results of bivariate regression analysis showed a strong positive correlation between UVB and UVA MEDs, Table 3 indicates that this particular combination is very unlikely in normal subjects ($P < 0.01$), and might be more in keeping with a drug-induced photosensitivity. We have described¹² the results of phototesting in a female patient who, a few months after taking naproxen for osteoarthritis, developed an increased erythral sensitivity to sunlight. Her UVB (300 ± 5 nm) and UVA (350 ± 30 nm) MEDs were 56 mJ/cm² and 14 J/cm², respectively. Three weeks after treatment with naproxen was stopped, the response to UVB was unchanged, but the MED at 350 nm had increased to 28 J/cm². This example illustrates how, on both occasions, the UVB and UVA MEDs were within their respective normal ranges (Table 2), yet the results of bivariate regression analysis (Table 3) would support the diagnosis of a drug-induced photosensitivity.

Finally, region 4 represents those subjects with a normal UVA MED accompanied by an unusually low UVB MED. Ramsay and Black¹³ reported such a group of patients, usually with long-standing eczema in whom photosensitivity developed that was confined to the UVB waveband. They called the condition 'photosensitive eczema' and separated it from other forms of chronic actinic dermatitis, such as actinic reticuloid, on the basis that the action spectrum was confined to wavelengths less than 320 nm. In a study of 34 male subjects suffering from chronic photosensitivity dermatitis,¹² it was found that the action spectrum for abnormal morphological responses was confined to the UVB waveband in only four cases. We reviewed the UVB (300 ± 5 nm) and UVA (350 ± 30 nm) MEDs in 88 patients with chronic actinic dermatitis that we have investigated and found that in no case did a patient exhibit that combination of UVA and UVB MED which would place him in region 4 in Table 4. Our findings are supported by a recent review,² which states that there are no separate groups within the syndrome of chronic actinic dermatitis in which the photosensitivity is confined to the UVB waveband.

It is clear that we would expect 95% of normal subjects in our local population to have a combination of UVA and UVB MEDs that fell within region 3 in Table 4. The converse, that a subject whose MEDs fell within region 3 is photobiologically normal, is not necessarily true. In Figure 1 the ellipse given by the solid line encompasses our 95% confidence intervals for normal subjects. It is defined by setting $P = 0.05$ in Equation 7 (see Appendix) and calculating all combinations of UVA and UVB MED that satisfy the equation. The points represent the UVA and UVB MEDs that we have obtained in 254 normal subjects (Fig. 1a); 114 patients with polymorphic light eruption (Fig. 1b); 88 patients with chronic actinic dermatitis (Fig. 1c); and 85 patients with a drug-induced photosensitivity (Fig. 1d). In plotting the graphs we have randomly assigned a given MED to lie between the observed value and the next lowest dose in the incremental series. This is done purely for illustrative purposes, so that each subject with a given UVB/UVA MED combination could actually be plotted separate from all the other subjects with the same combination. In each of the three photodermatoses it may seem that there is a significant number of subjects whose MEDs fall within the boundary of the ellipse. This is

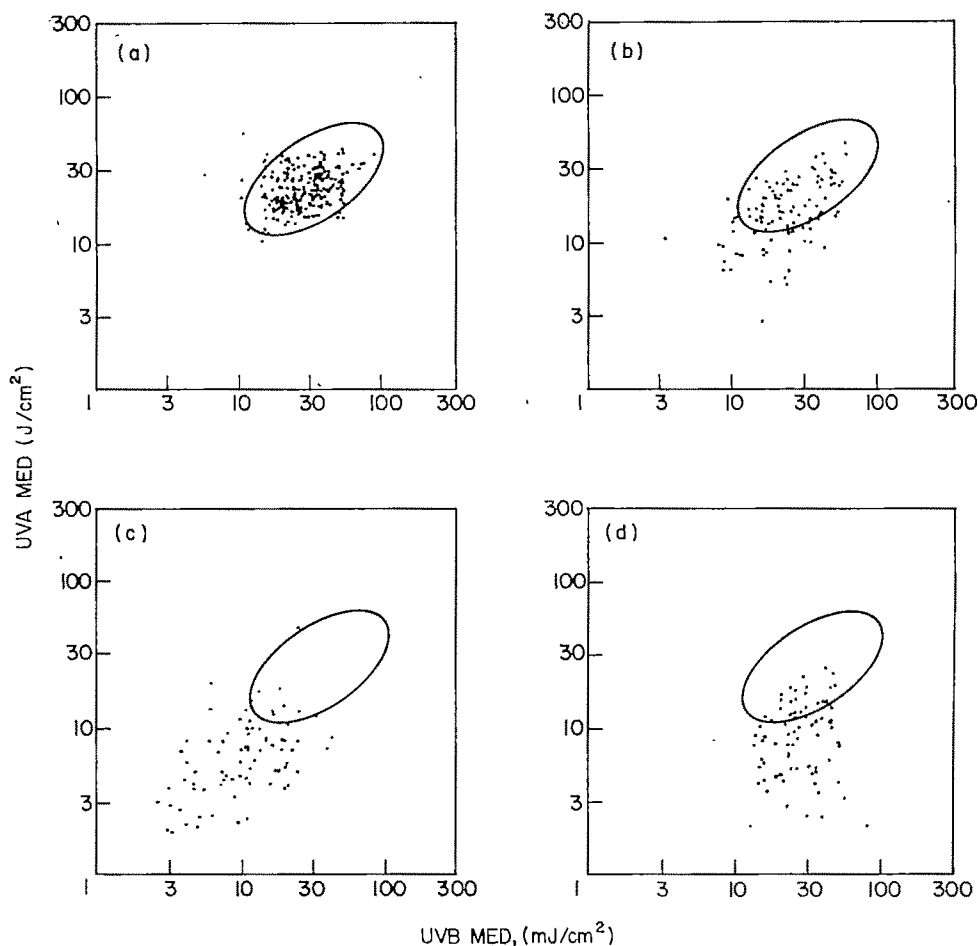


FIGURE 1. UVA (350 ± 30 nm) and UVB (300 ± 5 nm) MEDs observed in 254 normal subjects (a) 114 patients with polymorphic light eruption (b) 88 patients with chronic actinic dermatitis (c) and 85 patients with a drug-induced photosensitivity (d). The solid ellipse encompasses 95% of the normal subjects.

particularly so in polymorphic light eruption (PLE) where 84 of the 114 patients have 'normal' UVA and UVB MEDs. This observation is not surprising and supports the experience of others¹⁴⁻¹⁸ that the MED is usually normal in patients with PLE.

In conclusion, the 'normal range' in diagnostic phototesting should be viewed with circumspection. It can be misleading to regard erythral sensitivity as either normal or abnormal, and on the basis of a 'normal' MED disregard the possibility of an abnormal erythral response in a given subject.

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APPENDIX

The mean natural logarithm (μ_x) of the UVB MEDs is defined as

$$\mu_x = \frac{\sum_i \sum_j f_{ij} x_i}{\sum_i \sum_j f_{ij}} \quad (1)$$

where f_{ij} is the number of subjects with a log (UVB MED) of x_i and log (UVA MED) of y_j . The denominator,

$$\sum_i \sum_j f_{ij},$$

is simply equal to the total number of subjects (n ; $n = 254$).

Similarly the mean logarithm of the UVA MEDs, μ_y , is

$$\mu_y = \frac{\sum_i \sum_j f_{ij} y_j}{n} \quad (2)$$

Since the number of subjects (n) is large, $n \approx n-1$, and so the variances, σ_x^2 and σ_y^2 , may be adequately defined as

$$\sigma_x^2 = \left[\frac{\sum_i \sum_j f_{ij} x_i^2}{n} \right] / [n-1] - [\mu_x]^2 \quad (3)$$

$$\sigma_y^2 = \left[\frac{\sum_i \sum_j f_{ij} y_j^2}{n} \right] / [n-1] - [\mu_y]^2 \quad (4)$$

and the sample correlation coefficient (r) defined as

$$r = \left\{ \left[\sum_i \sum_j f_{ij} x_i y_j \right] / [n-1] - \mu_x \mu_y \right\} / [\sigma_x \sigma_y] \quad (5)$$

The median UVB MED is $\exp(\mu_x)$ and the 95% confidence interval defined as $\exp(\mu_x - t_{v,0.05}\sigma_x)$ to $\exp(\mu_x + t_{v,0.05}\sigma_x)$. Similar expression give the median UVA MED and 95% confidence interval if μ_x and σ_x are replaced by μ_y and σ_y , respectively. The term $t_{v,0.05}$ is the tabulated 5% point of the t distribution on $v = n-1$ degrees of freedom. When $n = 254$, $t_{v,0.05} \approx 1.96$.

We can examine the degree of correlation between the UVB and UVA MEDs by calculating the t -statistic defined as

$$t = r\{(n-2)/(1-r^2)\}^{\frac{1}{2}} \quad (6)$$

and to use t tables on $n-2$ degrees of freedom to test the significance of r .

Finally, the probability that a subject with a log (UVB MED) of x , and a log (UVA MED) of y , belongs to our 'normal' population is given by

$$P = \exp \{ - [(x - \mu_x)^2 / \sigma_x^2 - 2r(x - \mu_x)(y - \mu_y) / \sigma_x \sigma_y + (y - \mu_y)^2 / \sigma_y^2] / [2(1 - r^2)] \} \quad (7)$$

Inhibition of proliferation of HeLa cells by dithranol (anthralin) and 10-butyryl dithranol (butantrone)

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SUMMARY

The effects were studied of two antipsoriatic drugs, dithranol (anthralin) and butantrone on the proliferation of HeLa cells. Non-confluent monolayers of HeLa cells were treated with various concentrations (10^{-5} – 10^{-8} M) of dithranol or butantrone for 45 min. The cells were then cultivated for up to 48 h and treated with 10 μ M bromodeoxyuridine (BrdU) and 10 μ M fluorodeoxyuridine for 30 min before harvesting. Both drugs at concentrations of 10^{-6} and 10^{-5} M inhibited the BrdU uptake of HeLa cells, but did not cause any significant change in the proportion of cells in S-phase. The inhibitory effect of dithranol on cell number was linear with concentration 24 h after incubation. The highest (10^{-5} M) concentration of butantrone caused an abrupt decrease in cell number.

Dithranol (anthralin) is an effective drug for topical treatment of psoriasis. However, its side-effects, i.e. staining and irritation of the healthy skin, limit its use. Many derivatives of dithranol have, therefore, been studied in order to find a compound with a similar antipsoriatic effect as dithranol but with less side-effects.¹ The most promising of the 10-acyl derivatives studied is 10-butyryl-dithranol or butantrone.²⁻⁶ Butantrone is not cleaved to dithranol, but directly oxidated to 1,8-dihydroxyanthraquinone and is, therefore, not a prodrug of dithranol.⁷

Numerous clinical and experimental trials have been conducted to understand the antipsoriatic effect of dithranol. Treatment of guinea-pig skin with dithranol decreases the mitotic rate of keratinocytes to about one third of that in untreated epidermis.⁸ This supports the hypothesis that dithranol has a cytostatic effect on epidermal cells. In cell cultures dithranol inhibits DNA-synthesis and repair in cultured human keratinocytes.⁹⁻¹¹ It also inhibits keratinocyte growth as judged by mitotic index.¹² Our earlier studies have shown that both dithranol and butantrone inhibit the proliferation of cultured human keratinocytes and mouse embryonal carcinoma cells.¹³

In the present study we have used HeLa cells, an epithelial cell line derived from a human

cervix carcinoma. The HeLa cells were chosen because of their rapid proliferation and epithelial origin. Using flow cytometry we have analysed the effect of dithranol and butantrone on the cell cycle and on the uptake of bromodeoxyuridine, a thymidine analogue. This method was chosen because it permits the simultaneous measurement of the cell cycle phase and DNA synthesis at a single-cell level.

METHODS

Antipsoriatic drugs

Dithranol (MW 226) and butantrone (MW 282) were obtained from Orion Pharmaceuticals (Helsinki, Finland). Various concentrations of both drugs (10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} M) were dissolved in dimethylsulphoxide (DMSO) immediately before each experiment.

Cell cultures

HeLa cells were cultured in Eagle's minimum essential medium supplemented with glutamine, 10% fetal calf serum, penicillin and streptomycin. For the experiments, 3×10^5 cells were seeded onto 5 cm diameter Petri dishes. After 2 days, the non-confluent cultures were incubated with different concentrations of either dithranol or butantrone in serum-free medium for 45 min. As a control, cells were incubated with an equal concentration of DMSO. Thereafter, the cells were washed with serum-free medium and cultivated in serum-containing medium for up to 48 h.

BrdU labelling and cell counting

At the end of each experiment the cells were labelled with 10 μ M bromodeoxyuridine (Brd U) (Sigma Chemical Co., St. Louis, MO, U.S.A.) and 10 μ M fluorouracil (FU) (Sigma) for 30 min at 37°C and washed twice with phosphate-buffered saline (PBS). FU inhibits cellular thymidine kinase, which diminishes thymidine utilization thus increasing the BrdU incorporation. Thereafter, the cells were disaggregated with 0.25% trypsin in PBS. An aliquot of the cells was counted with a Coulter cell counter. The rest of the cells were centrifuged for 10 min and fixed with 2% paraformaldehyde (PFA) in PBS for 30 min on ice. The cells were then washed twice with PBS. The DNA was partially denatured with 2 M HCl for 15 min at 37°C. The HCl was neutralized by washing twice in 0.05 M sodium tetraborate buffer, pH 8.5. The cells were then labelled with mouse monoclonal anti-BrdU (IU-1, a gift from Dr Frank Dolbeare, Lawrence Livermore Laboratories, Livermore, CA) and with fluoroisothiocyanate-labelled goat anti-mouse immunoglobulin.¹⁴ Finally propidium iodide (Sigma) was added at a final concentration of 10 mg/ml. After an incubation time of at least 30 min the red and green fluorescence were measured with a FACS IV (Becton Dickinson) flow cytometer. The excitation wavelength was 488 nm at 300 mW. The emission at 515–530 nm and above 560 nm were recorded.

Recovery of HeLa cells after treatment with dithranol or butantrone

The recovery of cells after treatment with the drugs was studied to determine whether the effects of the drugs were transient or permanent. The cells were cultured as described above and treated with 10^{-6} or 10^{-5} M dithranol or butantrone in serum-free medium for 45 min. Thereafter, the cells were subcultivated in serum-containing medium without drugs and the Petri dishes were examined under the microscope about twice a week for up to 3 weeks.

To exclude the possibility that the slow recovery of the cells after treatment with the highest concentrations of the drugs was due to the small number of cells left after treatment, 100, 1000

and 10 000 HeLa cells were seeded onto 5 cm Petri dishes in 5 ml serum-containing medium without incubation with dithranol or butantrone. The cells were then cultivated for 2 weeks and the monolayers on the Petri dishes examined for confluency.

RESULTS

Effect of the drugs on cell number

The drugs had no significant effect on the cell number, when measured immediately after treatment (not shown), whereas 24 h after incubation with 10^{-5} M dithranol the number of HeLa cells was only about 60% of that in the controls. The decrease was linear with concentration. The highest concentration of butantrone had an even more pronounced effect. The number of HeLa cells on the Petri dishes was only 10% of that in control cultures. After 48 h both drugs had about the same effect on the cell number, although the highest concentration of dithranol had a somewhat smaller effect than that of butantrone (Fig. 1) Treatment with both 10^{-5} M dithranol and butantrone caused the cells to detach after 48 h.

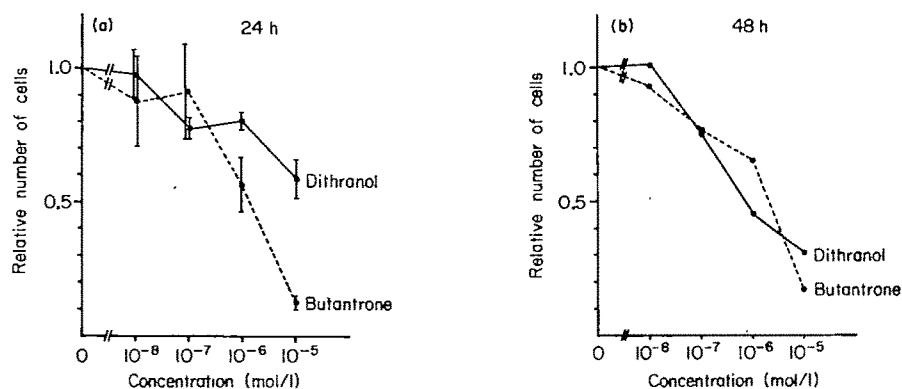


FIGURE 1. The effect of dithranol and butantrone on cell number (a) 24 h (four experiments) and (b) 48 h after incubation (two experiments). Bars indicate standard errors of the mean.

Recovery of cells

The HeLa cells treated with 10^{-5} M dithranol did not recover at all during the 3 weeks follow-up period. Some remaining, but apparently non-dividing cells were observed. The lack of proliferation could not be due to the small number of cells left after treatment, because after 1–2 weeks confluent monolayers had formed on Petri dishes seeded with only 100 cells. The cultures treated with 10^{-5} M butantrone showed some proliferation after 3 weeks (Table 1).

BrdU labelling and cell cycle

Dithranol and butantrone had no major effect on the percentage of S-phase cells at any of the concentrations studied (Fig. 2), not even after 48 h. At 24 and 48 h after treatment with 10^{-5} M butantrone, too few cells remained for any reliable measurement to be done.

No major changes in BrdU uptake were observed 15 min or 2 h after the incubation with dithranol or butantrone. After 24 h, both dithranol and butantrone at the highest concentration, 10^{-5} M, showed a clear inhibition of the BrdU uptake. After 48 h the inhibitory effect was more pronounced (Fig. 3).

TABLE 1. Recovery of HeLa cells 3 weeks after treatment with dithranol and butantrone (300×10^3 cells were treated with the drugs and then followed for three weeks.)

Drug	Concentration	Cell number
None		$630\,000\,000 \times 10^3$ *
Butantrone	10^{-6} M	$8\,100 \times 10^3$
Butantrone	10^{-5} M	600×10^3
Dithranol	10^{-6} M	32×10^3
Dithranol	10^{-5} M	33×10^3

* Minimum number of cells which would have been present if 300×10^3 cells had been cultivated for 3 weeks, properly dividing cultures, when they formed confluent monolayers (about 8×10^6 cells).

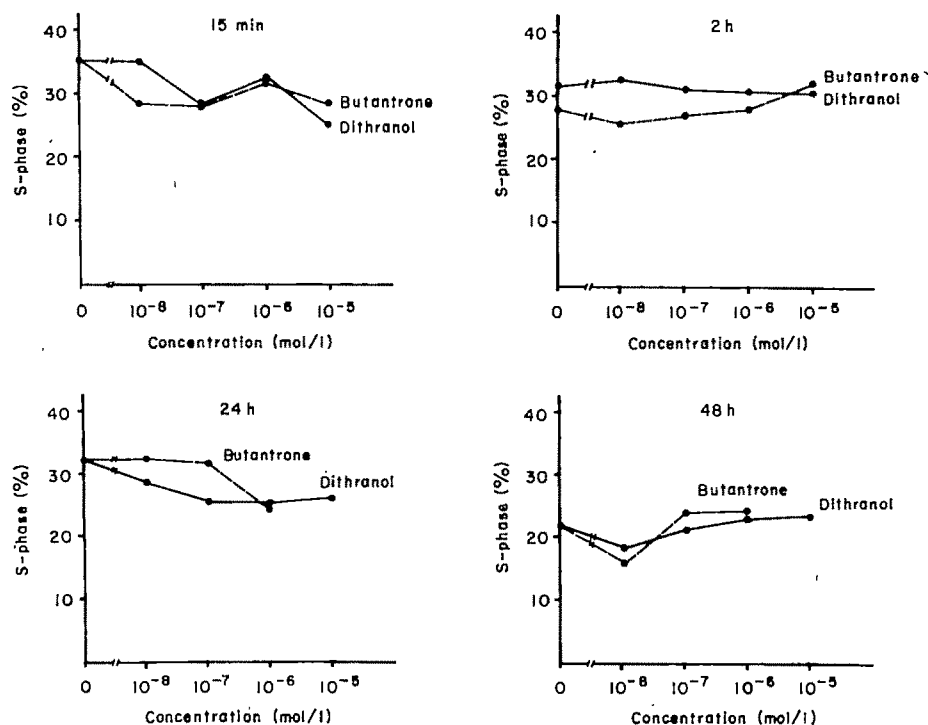


FIGURE 2. The effect of dithranol and butantrone on the cell cycle (S-phase) at different times after incubation with the drug.

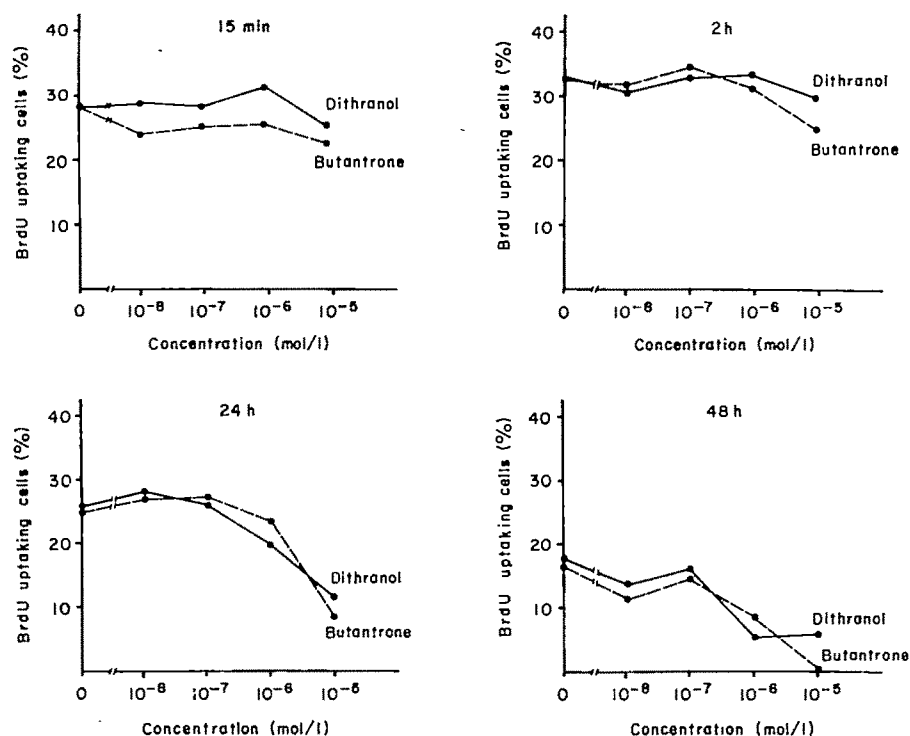


FIGURE 3. BrdU uptake of HeLa cells at different times after incubation with dithranol or butantrone.

DISCUSSION

In an undisturbed cell population, the number of cells that take up BrdU, equals the number of cells in S-phase. If the number of cells that take up BrdU is smaller than the number of cells in S-phase, some cells in S-phase do not actively synthesize DNA. If the drugs only affected cells in S-phase then cells in other parts of the cycle would continue until they reached S-phase, where they would accumulate.

The present study shows that dithranol and its 10-acyl derivative butantrone have a dose-dependent inhibitory effect on the proliferation of cultured HeLa cells. Both drugs at the concentrations of 10^{-6} M and 10^{-5} M inhibit the BrdU uptake of HeLa cells, but do not cause any major change in the proportion of cells in S-phase, or in G₁- and G₂-phase (not shown) as determined by cell cycle analysis with flow cytometry. The lack of accumulation of cells in S-phase indicates that the drugs do not have a specific effect on cells in S-phase, but affect cells in all parts of the cell cycle.

The effect of the drugs on BrdU uptake could not be seen until 24 h after incubation. The effect was even more pronounced after 48 h. Butantrone had a greater effect than dithranol on the number of cells in the cultures, especially after 24 h of culture, but also after 48 h. Dithranol seemed to be more toxic than butantrone to the cells, as HeLa cells did not recover after treatment with 10^{-5} M dithranol, whereas after treatment with 10^{-5} M butantrone there was a slow recovery and some cells started to proliferate.

We have earlier shown that dithranol at a concentration greater than 10^{-7} M gradually inhibits

DNA-synthesis of cultured human keratinocytes and mouse F9 embryonal carcinoma cells.¹³ Similar results have been reported by other investigators.⁹⁻¹² Few studies have been done on the effect of butantrone on cultured cells. Ten times as much butantrone as dithranol was needed to inhibit thymidine incorporation and cell growth in human keratinocytes and mouse embryonal carcinoma cells.¹³ The inhibitory effect of butantrone was not linear with concentration, but at a concentration higher than 10^{-6} M it seemed to be toxic. The finding is in keeping with our present results.

Jacques and Reichert have shown that another 10-acyl-derivative of dithranol, 10-acetyldithranol also has an abrupt inhibitory effect on human fibroblasts *in vitro*.¹⁵ The stronger effect of 10-acyl-derivatives compared with dithranol may be based on the longer 10-acyl substituent of the drugs, which has an increased affinity to the acyl chains of the phospholipids in the hydrocarbon core of the membrane bilayer. It is possible that, when the saturation is exceeded, the plasma membrane damage becomes irreversible and results in death of the cell.

The decrease in cell number after treatment with butantrone was greater than expected on the basis of its effect on BrdU uptake. After 24 h the number of cells on Petri dishes treated with 10^{-5} M butantrone was only 10% of that in control cultures. However, the number of cells that incorporated BrdU, only decreased from 25% to 9%. A similar, although less pronounced, result was obtained when cells were treated with dithranol. This demonstrates that these drugs not only affect cell proliferation, but also cause cell detachment. It could be expected that they cause changes in the cytoskeleton and/or other cytoplasmic structures of the cell. Preliminary results have shown a marked change in the organization of cytokeratin of HeLa cells following treatment with high concentrations of dithranol and butantrone.

At 24 and 48 h after treatment with both dithranol and butantrone the proportion of DNA-synthesizing cells decreased greatly as judged by the diminished BrdU uptake. This suggests that both drugs increase the cell cycle time of HeLa cells. Dithranol also increased the length of S and G₂ phase in rat epidermal cells *in vivo*.¹⁶ This effect may partly explain the antipsoriatic effect of the drugs, as the germinative cell cycle time is shortened in psoriatic keratinocytes.¹⁷⁻¹⁹

In clinical use dithranol is an effective and safe antipsoriatic drug when applied either in paraffin sticks or in white petrolatum, as in short contact therapy, although local irritation of the healthy skin is frequently observed. When butantrone is used in white petrolatum, even in high concentrations, there is less irritation of the skin.⁶ Used overnight in paraffin sticks butantrone causes irritation in some patients.²⁰ This may be due, as suggested by our cell cultures, to a toxic effect and an accumulation of the drug in the keratinocytes, which leads to their death.

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Identification of the epidermolysis bullosa acquisita antigen by LH 7·2 monoclonal antibody: use in diagnosis*

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SUMMARY

The sera from two patients with epidermolysis bullosa acquisita were blotted against dermal extracts in comparison with the mouse monoclonal antibody LH 7·2. This antibody reacts with carboxy terminal region of type VII collagen. The epidermolysis bullosa acquisita antisera showed binding to the same molecular weight protein as LH 7·2 confirming that the target antigen for epidermolysis bullosa acquisita antibodies is the carboxy terminal region of type VII collagen. This newly described collagen forms the major component of anchoring fibrils. These findings are consistent with established ultrastructural data which have shown that the epidermolysis bullosa acquisita antigen is located within and below the lamina densa. The monoclonal antibody LH 7·2 provides an internal standard for epidermolysis bullosa acquisita autoantiserum activity. The use of immunoblotting of epidermolysis bullosa autoantiserum in comparison with the monoclonal antibody LH 7·2 provides definitive investigation for the diagnosis of this disorder.

Immunoblotting data have shown that circulating epidermolysis bullosa acquisita (EBA) autoantibodies are directed against a unique basement membrane protein.¹ We have used protein blotting to compare the binding of EBA sera to that of the well-characterized monoclonal antibody LH 7·2^{2,3} and this has enabled us to establish the identity of the EBA antigen.⁴

EBA is a distinct immunobullous disorder which is diagnosed according to several criteria.⁵ These include: mechanobullous lesions which heal with scarring, no family history of bullous diseases, a subepidermal blister histologically with deposition of IgG at the dermo-epidermal junction and in the upper dermis on immunoelectron microscopy. Immunoblotting techniques

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provide the definitive investigation for the diagnosis of EBA. This has led to the recognition that although EBA is an immunochemically distinct disorder its clinical manifestations are broad and clinical criteria cannot be depended upon for the diagnosis of EBA.⁶ We report two cases which illustrate the clinical diversity of this disorder. Although immunoblotting techniques provide a specific investigation for the diagnosis of EBA, their use depends upon the ability to demonstrate that the sera binds to a protein of a specific molecular weight. For technical reasons this data may be difficult to reproduce. In this report we demonstrate how the use of protein blotting of EBA sera in comparison to the monoclonal antibody LH 7.2 may provide a reliable investigation for the diagnosis of EBA.

METHODS

Case histories

Case 1. A 71-year-old woman was referred to the Department of Oral Medicine at the London Hospital with a 2-month history of oral ulceration. For 2 weeks she had also had blistering of the skin. She gave no history of skin fragility. Examination showed blisters on both lips and on the alveolar ridge. Erosions were present on the palate and the buccal mucous membrane. Examination of the skin showed tense blisters and erosions on the upper trunk, arms and face. The following investigations were normal or negative: full blood count, erythrocyte sedimentation rate, urea and electrolytes, liver function tests, chest X-ray, serum protein electrophoresis, plasma glucose and antinuclear antibody. A biopsy taken from a blister on the oral mucosa showed a subepithelial bulla containing neutrophils. There was a mixed inflammatory infiltrate present subepithelially. Direct immunofluorescence on perilesional mucosa showed linear IgG, IgM, IgA and C3 at the basement membrane zone. Direct immunofluorescence on normal skin showed linear IgG, IgM, IgA and C3 at the basement membrane zone.

The clinical, histological and immunofluorescence findings were considered compatible with a diagnosis of bullous pemphigoid. The patient's disease failed to respond to treatment with prednisolone. Control of the disease was achieved with prednisolone 40 mg, azathioprine 100 mg and dapsone 150 mg. The blisters healed with scarring and milia formation and the patient has since noticed that blisters occur at sites of trauma. In view of this history a diagnosis of EBA was considered and further investigations were carried out. Indirect IgG immunofluorescence on 1 M sodium chloride split skin showed antibodies to a titre of 1 in 640 bound to the dermal side of the split. Indirect immunoelectron microscopy showed IgG deposits below the basal lamina.

Case 2. In 1979 this 25-year-old man presented with a widespread vesicular excoriated eruption. He gave no history of skin fragility. In 1968 he had developed epilepsy and was treated with carbamazepine, primidone and sodium valproate. Histology showed an intra-epidermal vesicle containing neutrophils. Direct immunofluorescence on perilesional skin showed linear IgA, IgG and C3 at the basement membrane zone. No diagnosis was made but the patient was treated with dapsone to which he responded well, discontinuing treatment after 1 year. Subsequently, he developed occasional bullae but required no systemic therapy until 1986 when he developed large numbers of bullae and erosions on his limbs and face which healed with milia formation. Histology showed a subepidermal bulla containing neutrophils. Peripheral to this lesion microvesicles also contained neutrophils. The dermis deep to the bulla contained a perivascular chronic inflammatory cell infiltrate. Direct immunofluorescence showed linear IgG, C3 and patchy IgA at the basement membrane zone. The patient was initially treated with 150 mg dapsone and failed to respond. The addition of 100 mg prednisolone and 150 mg

azathioprine induced healing. He was referred to the Department of Dermatology at St Thomas' Hospital for further investigation. Preliminary investigations showed the following results: haemoglobin 11.7 g %, mean corpuscular volume 100 fl, erythrocyte sedimentation rate 46 mm in 1 h. A bone marrow aspirate showed slight megaloblastic changes, compatible with azathioprine therapy. The following investigations were normal or negative: urea and electrolytes, serum folate and B₁₂, serum iron, total iron binding capacity, antinuclear antibodies, porphyrin screen, serum electrophoresis, barium meal. Indirect immunofluorescence showed IgG anti-basement membrane zone antibodies which bound to the base of sodium chloride split skin. Direct immunoelectron microscopy on perilesional skin showed IgG deposits below the lamina densa.

Western immune blots

Tissue preparation. Extracts of human skin basement membrane zone (BMZ) were prepared by incubating the skin in 1 M sodium chloride containing 50 μ M phenylmethylsulphonylfluoride (PMSF) at 4°C for 72 h, then removing the epidermis. A basement membrane preparation was made from both the epidermal and dermal fragments by incubating them separately in 8 M urea containing 0.3 M 2-mercaptoethanol and 50 μ M PMSF overnight.

BMZ preparations required for collagenase digestion were dialysed against 10 mM trishydroxymethylaminomethane (TRIS), pH 6.8. The dialysed BMZ extract was incubated with 1 unit of purified bacterial collagenase in the presence of 1 μ M calcium chloride.

Electrophoresis. Prior to SDS polyacrylamide gel electrophoresis (SDS-PAGE), samples were incubated in SDS sample buffer containing 10 mM TRIS pH 6.8, 100 mM dithiothreitol and 10% SDS at room temperature for 30 min. Proteins were subjected to SDS-PAGE according to Laemmli.⁷ The resolved proteins were transferred onto nitrocellulose⁸ and the resultant blots blocked overnight at 4°C in phosphate buffered saline (PBS), pH 7.4 (0.15 M sodium chloride, 0.02 M sodium phosphate) containing 3% bovine serum albumin (BSA) and 0.02% sodium azide.

Antisera. Blotting patterns of two EBA sera (Patient 1 and 2) were compared to normal human sera, a single bullous pemphigoid (BP) autoantiserum and LH 7.2, a monoclonal antibody to the carboxy terminal end of type VII collagen.^{2,3} Immediately before use the autoantisera were diluted 1:100 with PBS containing 3% BSA and the monoclonal antibody was diluted 1 in 5.

Probing. The nitrocellulose strips were probed with the primary antibody. The blots were then incubated with the appropriate peroxidase conjugated second antibody, rabbit anti-human IgG (Dako-Patts, Glostrup, Denmark) for the autoantiserum or rabbit anti-mouse immunoglobulins for the mouse monoclonals (Dako-Patts, Glostrup, Denmark). Antibody binding was detected by reacting the blots with PBS containing 0.5 mg/ml diaminobenzidine and 0.02% hydrogen peroxide.

RESULTS

Epidermal and dermal BMZ preparations were run in parallel and probed with the autoantiserum and LH 7.2. Using globular polypeptides as markers in electrophoresis the BP autoantiserum detected an antigen with a molecular weight of 220 kD on the epidermal preparation (Fig. 1) but the EBA autoantiserum and LH 7.2 showed no reactivity. The EBA autoantiserum and LH 7.2

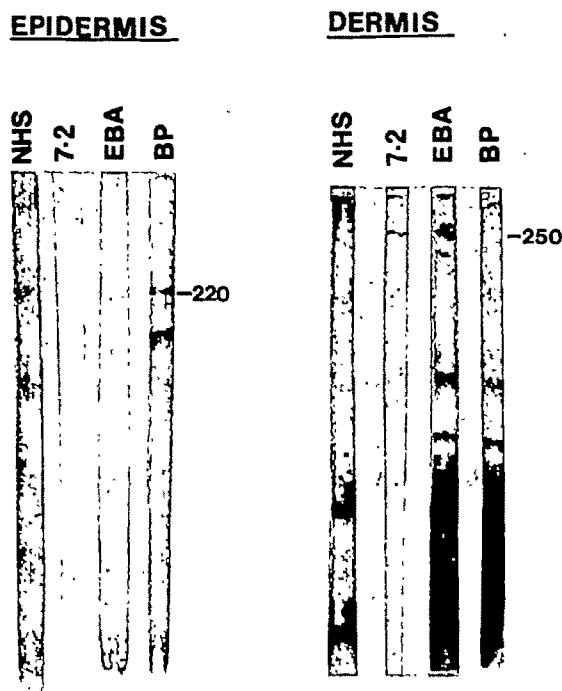


FIGURE 1. Epidermal and dermal BMZ preparations probed with BP and EBA sera (Patient 2), normal human serum (NHS) and the monoclonal antibody LH 7-2. The BP serum reacts with a protein in the epidermal extract at 220 kD and shows no reactivity with the dermal extract. The EBA serum reacts with a protein at 250 kD in the dermal extract and shows no reactivity with the epidermal extract. Normal human serum shows no reactivity. High non-specific binding in dermal blots below 97 kD reflects the large amount of dermal extract loaded in order to detect small amounts of type VII collagen.

detected an antigen at 250 kD with the dermal preparation (Figs 1 and 2), whereas the BP sera showed no reactivity (Fig. 1). Control normal human sera showed no reactivity with either preparation. Treatment of the dermal extract with purified bacterial collagenase digests the collagenous region of type VII collagen leaving the carboxy terminal region intact. Treatment with collagenase shifts the pattern of LH 7-2 reactivity from 250 kD to 145 kD. After collagenase digestion of the dermal extract, the EBA autoantisera also detect 145 kD protein (Fig. 2).

DISCUSSION

Immunoblotting techniques using circulating anti-basement membrane antibodies from sera of patients with EBA have shown that EBA autoantibodies are directed against a 290,000 Da protein, which is distinct from other known proteins of the basement membrane.¹ By comparing the activity of EBA autoantibodies with that of the recently characterized monoclonal antibody LH 7-2 on protein blots we have been able to identify the EBA antigen as the carboxy terminal region of type VII collagen, thus establishing the structure and function of this antigen within the basement membrane.⁴ Woodley *et al.* have recently published similar findings showing that EBA antigen is the carboxyl terminus of type VII procollagen.⁹ Ultrastructural studies have shown that type VII collagen comprises the major component of the anchoring fibrils.¹⁰ Type VII collagen is a newly described collagen which has a triple helical collagenous core and large

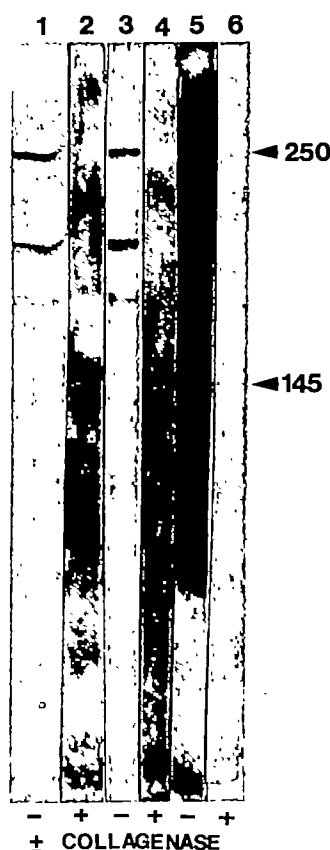


FIGURE 2. EBA sera reacted against dermal BMZ extracts in comparison with the monoclonal antibody LH 7-2. Both patients' sera (lanes 3 and 5) and LH 7-2 (lane 1) show binding to a protein of 250 kD. The band seen at approximately 210 kD is a breakdown product of type VII collagen which was not seen with concurrently run NHS. Treatment of the dermal extract with collagenase shifts the pattern of reactivity; after treatment the EBA sera (lanes 4 and 6) lane LH 7-2 (lane 2) react with a protein of 145 kD, corresponding to the molecular weight of the carboxy terminal region of type VII collagen.

flanking non collagenous regions.^{11,12} The tissue form is thought to be an antiparallel dimer with the carboxy terminal regions inserting into the inferior border of the lamina densa and into the anchoring plaques within the dermis.¹³

The finding that EBA antigen is type VII collagen is in agreement with previous data. The tissue distribution of type VII collagen is restricted to the basement membrane underlying stratified squamous epithelia and ectodermally derived glands,² findings which have been confirmed for the EBA antigen.^{14,15} Both cultured dermal fibroblasts and keratinocytes have been shown to synthesize EBA antigen.¹⁶ The synthesis of type VII collagen by fibroblasts has been demonstrated¹⁷ and we have been able to detect type VII collagen in the culture supernatant of cultured keratinocytes (unpublished observation). Ultrastructural studies have shown that the molecular target for EBA autoantibodies is located within and immediately below the lamina densa of the basement membrane of normal human skin¹ which concurs with the finding that the target antigen is the type VII collagen component of the anchoring fibril.

Using immunoblotting techniques we have found that both EBA sera and LH 7·2 bind to a basement membrane peptide of molecular weight 250,000 Da. Woodley *et al.*⁹ have shown that the molecular weight of EBA antigen is 290,000 Da. We accept that molecular weights above the 200,000 Da standard are difficult to resolve accurately and this may give rise to differences in the molecular weight obtained. However, the 290,000 Da molecular weight for the EBA antigen has recently been obtained by immunoblotting EBA sera on type VII procollagen which is derived from epithelial cultures.⁹ Our blotting data are obtained by using dermal extracts. Recent evidence suggests that the tissue form of type VII collagen lacks the aminoterminal globular domain and may, therefore, be of lower molecular weight than the biosynthetic form of type VII collagen¹⁸ and this may account for the lower molecular weight that we have observed. To date the use of immunoblotting in the investigation and study of EBA has depended upon autoantibody recognition of a protein of a specific molecular weight. Conditions for polyacrylamide gel electrophoresis such as choice of standards and preparation of substrate may vary from laboratory to laboratory; thus the same antigen may be ascribed different molecular weights in different laboratories. The use of LH 7·2 provides an internal standard on Western blotting for the identification of EBA autoantisera and this technique can, therefore, be used as the definitive investigation for the diagnosis of this disorder.

Criteria for the diagnosis emphasize the acquired mechanobullous nature of the disease.^{5,19} Recently two distinct clinical patterns of EBA have been recognized.²⁰ The classical presentation is of a non-inflammatory bullous disease with acral distribution which heals with scarring and milia formation. The alternative presentation is an inflammatory type in which there is a widespread vesico-bullous eruption in which skin fragility is not usually a prominent feature. Both of the patients reported here appear to have presented with the inflammatory disease pattern which evolved into the classical clinical form of EBA. Their cases illustrate the difficulty of making a clinical diagnosis of EBA when the classical features of trauma-induced blisters which heal with scarring are absent. Patient 1 illustrates how the clinical presentation of EBA may simulate cicatricial pemphigoid and it is now recognized that although EBA is an immunochemically distinct disorder the clinical manifestation may show features of overlap with both cicatricial and bullous pemphigoid.⁶

Type VII collagen appears to be a target for a number of diseases of the dermo-epidermal junction. In recessive dystrophic epidermolysis bullosa anchoring fibrils are defective and the LH 7·2 antigen is missing from patients with this disease.²¹ LH 7·2 is useful in the antenatal diagnosis of this disease.²² Equally in EBA LH 7·2 has provided the identity of the EBA antigen and may also be a useful marker on immunoblotting for EBA antibody activity in sera. Such an investigative technique may further aid the clinical characterization of this disease.

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The pathogenesis of lichen planus

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SUMMARY

The histological features of lichen planus (LP) are characterized by typical epidermal changes with dermal lymphocytes that are mostly Ia positive T cells. In order to find out whether the primary event of LP is damage to basal keratinocytes or a delayed hypersensitivity reaction in which an as yet unidentified antigen activates T lymphocytes that destroy keratinocytes, we transplanted skin obtained from six patients with LP. Two millimetre punch and split thickness of grafts were obtained from involved and uninvolved areas from each patient and grafted onto nude mice. Biopsies were taken from the grafts at 14 and 21 days after transplantation for histological and immunofluorescence studies and after 6 weeks for Dopa incubation for melanocyte populations. A complete disappearance of the pathological changes of LP was found 21 days following grafting. An increased number of melanocytes was noted. This indicates that the pathogenesis of LP may not be due to an inherent change in the epidermal cells, but rather to the migration of cellular elements of the immune system.

Lichen planus (LP) is a well known benign skin process with a long course of activity lasting 8-12 months but without any systemic involvement. Association of cases with autoimmune disorders and lichen planus-like eruption have been well documented.¹ Recent studies have demonstrated immunologic involvement in the pathogenesis of the disease,^{2,3} but have failed to reveal the primary event.

Nude mice served as an important tool for identifying site of defect in various pathologic processes.⁴ Grafts obtained from psoriatic patients and grafted onto nude mice revealed the persistence of characteristic histologic features 6 weeks following transplantation.⁵ This observation may suggest the existence of a primary defect in the epidermis in psoriasis.⁶

Recently we described the disappearance of the characteristic abnormalities in skin grafts from areas of alopecia areata following their transplantation in nude mice.⁷ This supports the idea that there is a systemic immunological mechanism for the pathogenesis of alopecia areata.

In this study biopsy, specimens from patients with LP were grafted onto nude mice to determine the pathogenesis of the disorder.

METHODS

Patients

Six patients with established clinical and histological LP were investigated. They were all male, with an age range of 24–48 years. Four of the patients had typical clinical and histological features of LP. Two patients had the hypertrophic lesions of LP. Two millimetre punch biopsies and split thickness skin samples were obtained from each patient. The skin biopsies were cut with a dermatome knife adjusted to cut at the level of the dermo-epidermal junction. Biopsies of skin were obtained from both involved and uninvolved areas from each patient. The donor grafts were approximately 1.2–1.5 cm in diameter. The 2 mm punch biopsies as well as the split-thickness skin were used as grafts and were taken from the legs of the affected patients after local anesthesia with 1% lidocaine. Five separate punch biopsies were obtained from each patient, 2 were for grafting and 3 for the following test: immunofluorescence, histological examination, and Dopa staining.

Animals

Outbred Balb/c nude mice, 2–3 months of age, were used. The animals were obtained from the pathogen-free animal breeding facility at the University of Tel Aviv, and were raised in a pathogen-free animal facility at the Faculty of Medicine, Technion, Haifa.

Skin grafting

Each 2 mm graft was inserted through a small incision in the skin into the subcutaneous tissue of the mouse's skin over the lateral thoracic cage. The split thickness skin graft was also transplanted at the same site. The graft sites were covered with petrolatum impregnated gauze and covered with standard adhesive bandage that was surgically stapled to the ventral surface of the animal.

Histology and immunofluorescence

Biopsies for routine light microscope examination were fixed in formalin and paraffin-embedded and from each specimen sections were cut and stained with haematoxylin and eosin. Cryostat sections (6 μ m) of skin were prepared for immunofluorescence using standard techniques. The reagents used were fluorescein labelled goat anti-human IgG, IgA, or IgM anti-complements C₃, C₄ and fibrin (Institut Pasteur Production, 92430 Marnes-la-Matignon, France).

Quantitative analysis of Dopa positive melanocytes

Skin biopsies were taken 6 weeks after transplantation and were evaluated for melanocytes density using Dopa incubation.⁸ The epidermis was separated by immersing the skin in 2 N sodium bromide for 45 min at 37°C. The epidermal sheets were then incubated with 0.1% Dopa (L-3, 4-dihydroxyphenylalanine) and 0.1 M of phosphate-buffered saline at a pH of 7.4 for 3–5 h at 37°C. The number of Dopa-positive melanocytes was estimated per mm² of skin.

RESULTS

Four of the patients demonstrated the typical clinical and histological features of LP. There was hyperkeratosis with focal hypergranulosis and irregular acanthosis with severe damage of the basal cell layer of the epidermis. There was a dense infiltrate of mononuclear cells in close

apposition to the epidermis and colloid bodies were observed in both the lower epidermis and upper dermis. Specimens obtained from two patients with hypertrophic LP demonstrated much more prominent acanthosis with papillomatosis and hyperkeratosis. Direct immunofluorescence in four patients showed colloid bodies that stained with IgM and fibrin. Fibrin deposition in a granular pattern at the dermo-epidermal junction zone was noted in three of the patients. The histological features 14 days after transplantation showed a reduction of the dense band-like inflammatory infiltrate but some of the epidermal changes seen before the transplantation were still apparent. The histology seen 21 days after grafting showed a complete disappearance of the pathological findings and the morphology of the skin appeared normal. However, hyperkeratosis was observed in some areas. Direct immunofluorescence showed none of the findings observed prior to transplantation.

Dopa incubation of separated epidermis showed a decreased number of melanocytes (119.25 ± 38) in the biopsies obtained from active lesions of the patients as compared with those from normal skin. Six weeks following transplantation there was a marked increase in the number of melanocytes (648.75 ± 146 , $P < 0.01$).

After 3 weeks there was no difference between transplants of normal and LP skin. Even the skin from hypertrophic of LP at that time appeared normal.

DISCUSSION

The observed disappearance of the histological abnormalities of LP following grafting onto nude mice, supports the notion that the typical changes seen in LP are immunologically mediated, most probably by the activated T cells in the upper dermis. We transplanted both split and full thickness skin obtained from each patient. The split grafts consisted mainly of epidermal cells whereas the full thickness also contained dermal components. The two types of grafts enabled us to differentiate whether the pathological process was due to a primary defect confined to the epidermis only or was associated with the dermis. The complete disappearance of all the characteristic histological and immunofluorescence features of LP in both types of grafts 3 weeks following grafts onto nude mice may negate the assumption for the existence of a primary defect in the epidermis of LP patients. The detection of many melanocytes in the epidermal sheet following grafting compared to the presence of only a few cells beforehand may strengthen the theory against there being a primary defect in the epidermis. So far the data on the fate of melanocytes in LP is not known. Most studies showed that the number of Dopa-positive melanocytes was markedly decreased, and that these cells were absent in the centre of active lesions.^{9,10} It is likely that the melanocytes were also involved and probably destroyed by the same process that affected the lower epidermal cells. Alternatively, the melanocytes were not destroyed but were not recognizable as they became Dopa-negative.¹⁰ Six weeks following the grafting the number and distribution of melanocytes in the grafted skin were normal.

Identification of the primary element involved in LP is crucial in understanding the pathogenesis of LP. So far there is conflicting evidence concerning the primary process that initiates the events leading to the histopathological changes of LP. Pinkus *et al.*¹¹ suggested that the epidermal basal cell layer is the primary part involved. Electron microscopy of the skin in LP shows damage of the cells in the basal layer which changes affects the desmosomes and hemidesmosomes.^{12,13} However, there is evidence that the primary event is not damage to the basal keratinocytes, but rather a delayed hypersensitivity response in which unidentified antigens, after being processed in the Langerhans cells, attracts T lymphocytes which presumably are able to act as effector cells against keratinocytes and have cytotoxic potential.¹⁴

The tissue damaging process is self-perpetuating, attracting more cells to the site of inflammation. This assumption is based on the observation that the dermal infiltrate consists mainly of T lymphocytes¹⁵⁻¹⁷ and an increased number of Langerhans cells in the epidermis in the earliest lesions.¹⁴ Furthermore, HLA-DR expression on keratinocytes was observed in a variety of skin diseases including lichenoid skin diseases and LP.¹⁸ A number of clinical and experimental observations suggest that HLA-DR⁺ keratinocytes may help to direct the movement of lymphoid cells towards the skin.¹⁹⁻²¹

Our study may indicate that the pathogenesis of LP is not due to an inherent change in the epidermal cells, but rather to the migration of cellular elements of the immune system which are antigenically driven. The disappearance of the epidermal defect associated with the detection of Dopa-positive melanocytes following grafting appears to support this concept.

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Pityriasis rotunda as a cutaneous marker of hepatocellular carcinoma: a comparison with its prevalence in other diseases

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SUMMARY

In an attempt to substantiate the claim that pityriasis rotunda may be a useful cutaneous marker of hepatocellular carcinoma in South African Blacks, the prevalence of the rash in 63 unselected South African Blacks with this tumour was compared to that in 63 matched patients with active tuberculosis, 63 with other malignant tumours, and 63 with various forms of chronic benign hepatic disease. The prevalence of pityriasis rotunda in hepatocellular carcinoma was 15.9%, which was appreciably greater ($P=0.0005$) than the overall prevalence of the rash (2.6%) in the controls. The prevalence was 4.8% for tuberculosis ($P=0.038$), 0% for other malignant diseases ($P=0.0007$), and 3.2% for chronic benign hepatic disease ($P=0.015$). We conclude that the presence of pityriasis rotunda is a useful pointer to the diagnosis of hepatocellular carcinoma in South African Blacks.

We recently suggested that pityriasis rotunda may be a useful cutaneous marker of hepatocellular carcinoma (HCC) in South African Blacks.¹ Because this rash is known to occur in association with a variety of chronic debilitating diseases including tuberculosis, malignant tumours, severe nutritional deficiency, and hepatic disease,²⁻⁵ our observation of an association between pityriasis rotunda and HCC was uncontrolled and, therefore, unproven.⁶ Support for the validity of the association would be provided if pityriasis rotunda could be shown to occur appreciably more often with HCC than with the other conditions known to be accompanied by the rash. We report here the results of such a comparison.

METHODS

Sixty-three South African Blacks with HCC were examined at Baragwanath Hospital over a 3-year period. These patients were unselected in so far that they comprised all of the HCC patients

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admitted to the general medical wards of the hospital during the period of the study. There were 55 men (mean age 55 years) and eight women (mean age 30 years). The preponderance of older males reflects the usual pattern of occurrence of HCC in an urban Black population. The diagnosis of HCC was confirmed histologically in 58 patients; in the remainder the diagnosis was made on the basis of a compatible clinical picture, the findings on hepatic imaging, and a serum α -fetoprotein concentration of greater than 500 ng/ml (normal less than 20 ng/ml). Three control groups, each comprising Black patients matched for age and sex with the HCC patients, were examined in the same wards of Baragwanath Hospital over the same period. The first consisted of 63 patients with active tuberculosis: 56 patients with pulmonary tuberculosis proven on sputum examination and chest X-ray findings; five with tuberculous pleurisy proven on pleural biopsy; and two with abdominal tuberculosis proven on laparoscopy and peritoneal or hepatic biopsy. All of these patients showed some degree of wasting, and the majority were severely wasted. The second group comprised 63 patients with malignant diseases other than HCC: 24 patients with bronchogenic carcinoma; 15 with gastrointestinal carcinomas (six oesophageal, four colonic, three pancreatic, two gastric); 10 with haematological malignancies (six with leukaemia, two with lymphoma, two with myeloma); four with carcinoma of the throat or larynx; three each with renal or prostatic carcinoma; three with sarcomas; and one with breast carcinoma. In each of these patients, the diagnosis was confirmed on biopsy or on cytological examination. The majority of these patients were obviously wasted. The third group of controls comprised 63 patients with chronic hepatic disease: 40 patients with alcohol-related hepatic disease, including 18 with established micronodular cirrhosis, 20 with severe haemosiderosis and portal fibrosis, and two with alcoholic hepatitis; seven with obstructive jaundice caused by chronic pancreatitis or strictures of the common bile duct; five with chronic active hepatitis; four with one or more amoebic hepatic abscesses; three with schistosomiasis, portal fibrosis and hypersplenism; three with chronic hepatic venous outflow obstruction (Budd-Chiari syndrome); and one with extensive amyloid infiltration of the liver. The diagnosis in these patients was made on clinical assessment aided by appropriate serological or radiological investigations, and confirmed by hepatic biopsy when indicated.

The data were analysed statistically using Fisher's Exact test, Student's *t*-test, or the Mann-Whitney *U*-test, where appropriate.

RESULTS

Pityriasis rotunda was present in 10 patients with HCC (15.9%) (SE of the proportion 4.5). Nine of these patients were men (mean age 58 years), and one was a 29-year-old woman. An analysis of the relative frequency of pityriasis rotunda according to age and sex is shown in Table 1. Although pityriasis rotunda appears to be more frequent in older patients, the difference was not statistically significant ($P=0.572$) (Mann-Whitney *U*-test). The apparently higher frequency rate in males did not reach statistical significance ($P=0.627$). Eight of the male patients also showed haemosiderosis and two were cirrhotic.

Pityriasis rotunda was observed in five of the total control population (2.6%) (SE of the proportion 1.1) ($P=0.0005$ vs. the HCC patients). Three of these patients suffered from tuberculosis, a prevalence of pityriasis rotunda of 4.8% (SE of the proportion 2.7) ($P=0.038$ vs. HCC) and two chronic benign hepatic disease (3.2%; SE of the proportion 2.2; $P=0.015$ vs. HCC). The three patients with tuberculosis and pityriasis rotunda were older patients (two men and one woman) (mean age of 61 years compared with 45 years in the remaining patients with tuberculosis; $P>0.05$) and each admitted to drinking traditional 'home-brewed' beer. Hepatic

TABLE 1. Relative frequency, by age and sex, of pityriasis rotunda among Black patients with hepatocellular carcinoma, tuberculosis, other malignant diseases and chronic hepatic diseases

	Age	With pityriasis rotunda				Without pityriasis rotunda			
		Male		Female		Male		Female	
		Number	%*	Number	%	Number	%	Number	%
Hepatocellular carcinoma	20-40	1	11	1	100	6	12	6	86
	41-60	3	33	—	—	20	44	1	14
	60+	5	56	—	—	20	44	—	—
	Total	9	100	1	100	46	100	7	100
Tuberculosis	20-40	—	—	—	—	7	14	7	100
	41-60	—	—	1	100	23	43	—	—
	60+	2	100	—	—	23	43	—	—
	Total	2	100	1	100	53	100	7	100
Other malignant diseases	20-40	—	—	—	—	7	13	7	88
	41-60	—	—	—	—	23	42	1	12
	60+	—	—	—	—	25	45	—	—
	Total	0	0	0	0	55	100	8	100
Chronic hepatic disease	20-40	—	—	—	—	7	13	7	88
	41-60	2	100	—	—	21	40	1	12
	60+	—	—	—	—	25	40	—	—
	Total	2	100	0	0	53	100	8	100

*To nearest whole number.

function tests and sonar examinations were normal and serum α -fetoprotein levels were not raised. However, serum ferritin concentrations were markedly raised (mean 3200 ng/ml; normal < 400 ng/ml), indicating the presence of haemosiderosis. Liver biopsies were not performed. The two patients with pityriasis rotunda and chronic hepatic disease were both elderly men (mean age 60 years compared with 49 years for the other patients in this control group; $P > 0.05$). Both had alcohol-related disease, one a micronodular cirrhosis, and the other haemosiderosis and cardiomyopathy. Of the patients with other malignant diseases, none of whom had pityriasis rotunda ($P = 0.0007$), the majority were severely undernourished and 10 showed, in addition, overt hepatic disease (obstructive jaundice in three patients with pancreatic carcinoma, hepatic metastases in five, and haemosiderosis in two). As most of these patients did not have a liver biopsy performed, the overall frequency of haemosiderosis and alcoholic hepatic disease is not known.

DISCUSSION

Pityriasis rotunda is a form of acquired ichthyosis that has been described in association with various debilitating diseases (Fig. 1),²⁻⁵ and whose cause remains uncertain. Early reports described pityriasis rotunda in Japanese,² South African Blacks^{1,3,4} and West Indians,⁷ but more recently the rash has been noted in American Blacks⁸ and in a Sephardic Jew.⁹

In this study, the prevalence of pityriasis rotunda in patients with HCC (16%) was significantly higher than that occurring in matched patients with tuberculosis, other forms of malignant disease, and chronic benign hepatic disease, supporting our belief that this rash is a

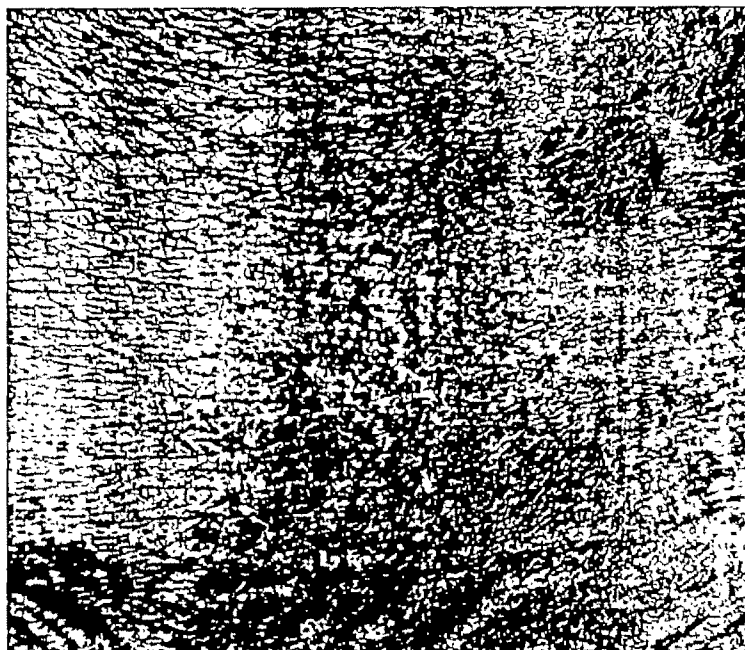


FIGURE 1. Pityriasis rotunda: close-up view of several lesions.

useful cutaneous marker of HCC in South African Blacks.¹ Swift and Saxe⁵ reported 63 South African patients with serious chronic illness and pityriasis rotunda. Because of the diversity of the associated illnesses, they concluded that nutritional disturbance was the likely aetiological factor. However, in the present study pityriasis rotunda did not appear to be attributable to poor nutrition *per se* as the majority of the controls with tuberculosis and other malignancies, and some of those with chronic hepatic disease were poorly nourished and yet did not have the rash. Moreover, pityriasis rotunda is seen in the early stages of HCC when the patients are not obviously undernourished or wasted. The patients with HCC were not more wasted or more undernourished than the controls with tuberculosis or malignant disease. Also there was no correlation with hepatic disease in general, the overall prevalence of pityriasis rotunda in patients with hepatic disease (including those with hepatic metastases) being 2.7%. There was no correlation between the presence of the rash and the hepatitis B virus status of the HCC patients (results not shown).

The relation between pityriasis rotunda and haemosiderosis is more difficult to assess. Haemosiderosis is common in older Blacks who have consumed large volumes of 'home-brewed' beer with a high iron content. Mild or moderate degrees of haemosiderosis do not cause overt hepatic disease. Excessive tissue iron was apparent on hepatic biopsy in 80% of the HCC patients with pityriasis rotunda, but also in 62% of HCC patients without this rash ($P=0.242$). The prevalence of haemosiderosis could not be assessed in the control groups as many did not have a liver biopsy performed. The frequent finding of haemosiderosis in patients with pityriasis rotunda may be related to two factors. Firstly, the rash was commonest in older patients, who are also most likely to have consumed traditional 'home-brewed' beer to excess (younger patients drink commercially prepared beer which is free of iron) and to be siderotic. Secondly, heavy drinkers are prone to develop both cirrhosis (and later HCC) and tuberculosis, all of which may be associated with pityriasis rotunda.

Although pityriasis rotunda occurred more often in older male patients in the present analysis, neither the sex difference nor the age difference was statistically significant. The rash is by no means confined to older patients or those with haemosiderosis, being seen in rural Blacks who often present with HCC in their twenties and thirties and when they do not have haemosiderosis (MCK, unpublished data).

We conclude that pityriasis rotunda is a useful cutaneous marker of HCC in South African Blacks, although it may also be seen in other debilitating diseases.

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Epidermal dysplasia and cyclosporine therapy in renal transplant patients: a comparison with azathioprine

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SUMMARY

The prevalence of dysplastic cutaneous lesions was determined in a group of 68 renal transplant patients whose immunosuppressive therapy included cyclosporine but not azathioprine. The mean age of the patients was 41 years (range 17–67), and the mean transplant time was 44 months (range 24–75). Dysplastic cutaneous lesions were found in 14 out of 68 patients, a prevalence of 20.6%. Although the lesions arose on sun-exposed sites, there was no apparent correlation with previous sun-exposure. The cumulative prevalence of dysplastic cutaneous lesions in 64 patients who had received cyclosporine for between 24 and 72 months was compared with 33 renal transplant patients who had been treated with azathioprine over a similar period of time. The overall prevalence of cutaneous dysplasia was 22% in the cyclosporine group and 9% in the azathioprine group. The cumulative prevalence of cutaneous dysplasia in the cyclosporine group was greater at all time points studied. In contrast to previous reports, we have found no evidence that the risk of cutaneous malignancy in patients treated with cyclosporine is less than that found in patients maintained on azathioprine.

It is now well recognized that renal transplant recipients whose immunosuppressive therapy includes azathioprine are at an increased risk of developing skin cancer.^{1,2} In recent years, cyclosporine has been used as an immunosuppressive agent in most transplant units and in some, cyclosporine and azathioprine are initially given concurrently, the azathioprine being later withdrawn. This type of drug regimen makes it difficult to establish the relative contribution of the individual drugs when later complications, such as cutaneous malignancy, develop.

Since 1982, a number of patients from this centre have been treated with cyclosporine, either alone or in combination only with prednisolone, following renal transplantation. These patients have never been exposed to azathioprine. This has enabled us to make a direct comparison of the rate at which dysplastic cutaneous lesions arise with two different treatment regimens.

The aim of this study was to determine the cumulative prevalence of neoplastic and pre-neoplastic skin lesions in a group of renal transplant recipients taking cyclosporine either alone

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or in combination with systemic steroids. We have compared the present study with the results of a previous one from this centre that dealt with the prevalence of cutaneous malignancy in renal transplant recipients immunosuppressed with azathioprine and prednisolone.

METHOD

In the first part of the study, 68 patients (45 males, 23 females) were examined; all patients had undergone renal transplantation at this centre and were taking cyclosporine either alone or in combination with prednisolone. The initial dose of cyclosporine was in the range of 8–15 mg/kg per day, and this was then adjusted according to blood levels, with the aim of maintaining a trough level of 300–700 ng/ml of whole blood (using the non-specific radio-immunoassay). Forty out of sixty-eight (59%) patients were taking prednisolone concurrently (5–15 mg/day). The mean age of the patients was 41 years (range 17–67 years), and the mean duration of immunosuppression was 44 months (range 24–75 months). Patients who were less than 24 months post-transplant were excluded from the study. This was to ensure that patients who had already developed pre-malignant or clinically unidentified malignant skin lesions at the time of transplantation were excluded. All patients were interviewed and examined by a dermatologist. Particular note was made of previous recreational and occupational sun-exposure. As in previous studies,^{3,4} patients were defined as having experienced a high degree of sun-exposure if they had worked for more than 5 years in outdoor employment or had spent more than 3 months in a tropical or sub-tropical region.

The nature and extent of any cutaneous lesions present were noted. Any skin lesion which was thought clinically to be malignant or pre-malignant was biopsied and submitted for histological analysis. A total of 35 biopsies were taken from this group.

RESULTS

Patients were assigned to one of two groups for analysis: Group 1, patients with histological evidence of cutaneous malignancy or pre-malignancy (dysplastic group); Group 2, patients with no evidence of cutaneous malignancy or pre-malignancy (non-dysplastic group). Of the 68 patients studied, 14 (11 males, 3 females) had dysplastic epidermal lesions, an overall prevalence of 20.6%. Twelve patients had solar keratoses, one patient had a solitary basal cell carcinoma and one patient had both solar keratoses and a basal cell carcinoma.

The mean age of the dysplastic group at the time of transplant was 53 (SD 10.2) years compared with 37.4 (SD 12.9) years for the non-dysplastic group.

Patients with dysplastic skin lesions had not been immunosuppressed for a greater time than patients in the non-dysplastic group (mean time since transplantation: dysplastic group: 36 (SD 9.1) months; non-dysplastic group: 47 (SD 16.2) months).

Nine out of 14 patients (64%) in the dysplastic group were taking prednisolone in addition to cyclosporine, the proportion being similar to that for the group as a whole. None of the patients had previously been treated with other immunosuppressive agents.

All the dysplastic lesions arose on sun-exposed sites (i.e. face and dorsum of hands). However, there was no statistically significant excess of patients with historically high levels of sun-exposure in the dysplastic group (9/14) when compared with the non-dysplastic group (22/54) ($\chi^2 = 2.48$, $P > 0.1$). This finding is similar to that found in our previous study in patients who were taking azathioprine.⁴

Comparative prevalence of cutaneous dysplasia in patients immunosuppressed with either azathioprine or cyclosporine

In a previous similar study of 85 renal transplant patients from this centre treated with azathioprine (0.75–2 mg/kg/day) and prednisolone (7.5–15 mg/day), we found dysplastic epidermal lesions in 25% of patients.⁴ In the present study this was 20.6%. These figures, however, are not strictly comparable, as the azathioprine group included many long-term survivors and 53% of the group had survived for more than 80 months post-transplant. Long-term survivors in the cyclosporine group were under-represented because of the relatively recent introduction of this drug into clinical practice. We therefore compared the cumulative prevalence of dysplastic lesions in the two groups only for patients who were between 24 and 72 months post-transplant. There were 33 patients in the azathioprine group, and 64 patients in the cyclosporine group within this time range.

The ages of the two groups were similar. In the azathioprine group, the mean age was 39 years and in the cyclosporine group 41 years. The proportion of patients with high sun-exposure was also similar (azathioprine group: 14/33 (42%), cyclosporine group: 29/64 (45%).

In the azathioprine group 3/33 (9%) had dysplastic lesions. Two patients had solar keratoses, and one patient had a basal cell carcinoma. This compares with 14/64 (22%) patients who had either solar keratoses or basal cell carcinoma in the cyclosporine group.

The cumulative prevalence of dysplastic lesions in the cyclosporine group is greater than that in the azathioprine group at all time intervals studied (Fig. 1) although the difference is only statistically significant for the time period 37–48 months post transplant ($\chi^2 = 5.27$, $P < 0.05$).

DISCUSSION

The findings of this study are preliminary and are based on relatively small numbers of patients. A clearer picture of the true risk of cyclosporine therapy will only emerge when the duration of immunosuppression is longer in the group as a whole. However, our results indicate that renal transplant recipients taking cyclosporine have a significant risk of developing cutaneous malignancy and pre-malignancy. The magnitude of this risk would appear to be at least as great as that of patients taking azathioprine, and our present study suggests that it may be greater. There are no reports from the U.K. relating to the prevalence of cutaneous malignancy in

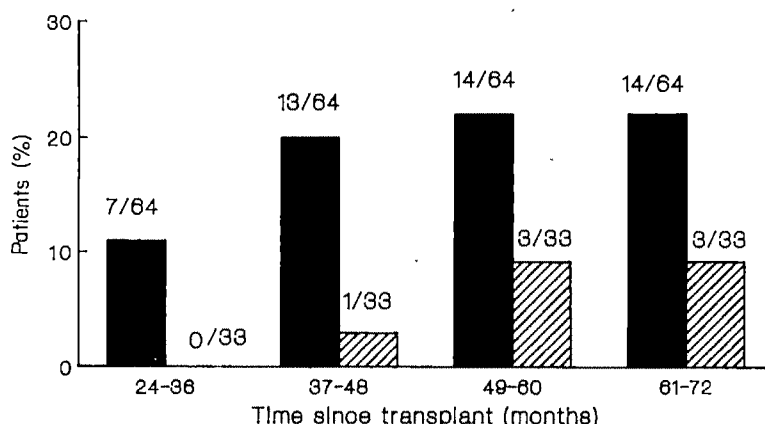


FIGURE 1. Cumulative prevalence of patients with dysplastic skin lesions following transplantation.
▨ Azathioprine group (n = 33), ■ Cyclosporine group (n = 62).

patients receiving cyclosporine alone following organ transplantation, although isolated cases where this complication had occurred have been described.^{5,6} The risk of developing cutaneous malignancy relative to the normal non-immunosuppressed population is impossible to quantify as accurate prevalence figures are not yet available in this country. However, in the relatively young age group assessed in this study, it is likely that dysplastic epidermal lesions would be encountered only very infrequently.

The findings of our study are in contrast to previous reports which have suggested that the risk of developing cutaneous malignancy is lower in patients treated with cyclosporine when compared to patients treated with azathioprine.⁷⁻⁹ Evaluation of these reports is difficult as they do not include details of previous immunosuppressive therapy, and it is not clear what proportion of patients have been treated with both azathioprine and cyclosporine, or have been changed from azathioprine to cyclosporine. This in itself, is thought to favour the development of skin cancer.¹⁰ Furthermore, the reports by Penn^{7,8} are based on data collected worldwide by the Cincinnati Transplant Tumour Registry and deal with a heterogeneous group of patients, from geographically disparate areas. None of the previous studies have reported the cumulative prevalence of cutaneous dysplasia in reasonably well matched series of patients from a single centre. In addition, previous comparative studies have not included patients with pre-malignant skin lesions, although it is recognized that these patients constitute a group at special risk of developing invasive cutaneous malignancy and, therefore, require especially careful follow-up. Although the rate of progression from *in situ* epidermal carcinoma to invasive malignancy appears to be low in the non-immunosuppressed individual,¹¹ our experience suggests that this is not the case for immunosuppressed individuals in whom invasive tumours occur relatively frequently, and at an earlier age. These factors may explain the discrepancy between our findings and those previously reported.

We feel that in view of our results, continued surveillance of the skin of patients taking cyclosporine following renal transplantation is necessary. We have no evidence, so far, that the risk of these patients developing cutaneous malignancy is less than in patients maintained on alternative immunosuppressive regimens. Whilst it would seem prudent to advise the use of sunscreens in this group of patients, there is as yet no clear evidence of a protective effect, and in our experience the correlation between previous sun-exposure and subsequent development of skin tumours is weak.

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Sunbed-induced pseudoporphyria

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SUMMARY

The clinical details of four fair-skinned women with recurrent blistering and skin fragility are presented. All had considerable sun-exposure and had used sunbeds for years. Specific causes of bullous dermatoses including drug ingestion, porphyria, cutaneous amyloidosis and epidermolysis bullosa acquisita were excluded. Long-term recurrent exposure to high doses of sunbed radiation in chronically sun-damaged skin appears to be a further cause of pseudoporphyria.

The immediate and long-term side-effects of prolonged sunbed predominantly UVA exposure are gradually being recognized. We describe a series of patients demonstrating blistering and skin fragility and which has recently been attributed to prolonged exposure to UVA.^{1,2}

CASE REPORTS

Case 1

A 28-year-old woman presented with a 3-year history of recurrent blistering and erosions on the limbs after minor trauma. She also had atopic eczema. She was on no medication. Despite being very fair-skinned, she had become a sun-worshipper, and had used sunbeds repeatedly over the previous three years. It was estimated that the total UVA exposure was in excess of 10 kJ/cm².

On examination, she had red hair and an unusual orange/brown tan with many freckles. The skin was dry with decreased elasticity and the appearance was that of a much older person. There were striking linear purpuric streaks (Fig. 1) and excoriations on the legs, arms and abdomen with groups of blisters on the limbs (Fig. 2). There were no milia. She had eczema on the sides of the neck.

The following investigations were normal: full blood count, clotting screen, Hess's test and plasma ascorbic acid, liver and renal function, cryoglobulins, immunoglobulins, protein electrophoresis, autoantibody screening. Quantitative porphyrin studies on urine, blood and stool were normal and fluorescence emission spectroscopy of the serum showed no detectable porphyrins.

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FIGURE 1. Extensive purpura on the legs; freckles and erosions are also evident (Case 1).

Histopathology of a blister from the arm showed a sub-epidermal split without any associated inflammation (Fig. 3). There was an increase in dermal connective tissue and an increased amount of PAS-positive hyaline material around superficial blood vessels. Marked solar elastosis was evident in the biopsy obtained from the leg. Congo red stain for amyloid on both the skin and rectal biopsies was negative.

Immunofluorescence showed weak dermo-epidermal basement membrane zone staining with IgG and fibrin but marked upper dermal perivascular deposits of IgG and fibrin. Indirect immunofluorescence of serum did not reveal circulating antibodies to basement membrane, intercellular or epidermolysis bullosa acquisita antigens. Monoclonal type IV collagen antibody studies showed a marked perivascular fluorescence (Fig. 4).

Electron microscopy showed a patchy reduplication of basement membrane at the dermo-epidermal junction and around blood vessels (Fig. 5). There was no evidence of amyloidosis as seen with electron microscopy. The site of splitting in the skin was below the lamina densa in the superficial papillary dermis (Fig. 6).

Irradiation monochromator testing showed a 4–5 fold increase in MED at 300 and 307.5 nm in keeping with considerable recent sun and sunbed exposure.

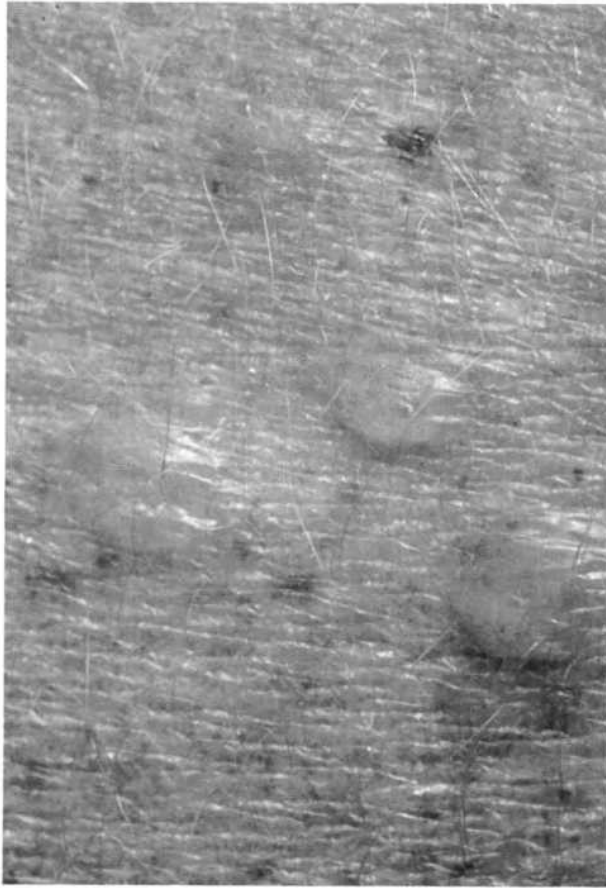


FIGURE 2. Grouped blisters arising on non-inflamed skin on the arm (Case 1).

Suction-blister time was assessed in view of the skin fragility and blistering. Suction cups were applied to the affected areas of skin at a negative pressure of 200 mm/Hg. The time of the appearance of blisters was 70 min which was within the normal range.

A toxicology screen for drugs reported to cause pseudoporphyria including naproxen,³ frusemide,⁴ dapsone,⁵ tetracycline^{5,6} and pyridoxine⁷ was negative.

Case 2

A 33-year-old woman, presented with a 13-month history of a blistering eruption involving the dorsa of both hands following trauma. She had noted increasing fragility of her skin and although the problem had improved during 1988, she had continuing blistering on the face and lower legs. She was on no drugs. Further questioning revealed that the patient had used a sunbed about once every 2 weeks for at least 8 years and more frequently in the previous 2 years. In the course of her work she travelled frequently to tropical countries where she sunbathed.

Examination of the skin revealed a very deep tan. There were no blisters or milia seen, but multiple scars were present at the sites of previous blistering on the lower legs.

The following investigations were performed: quantitative estimations of porphyrins on

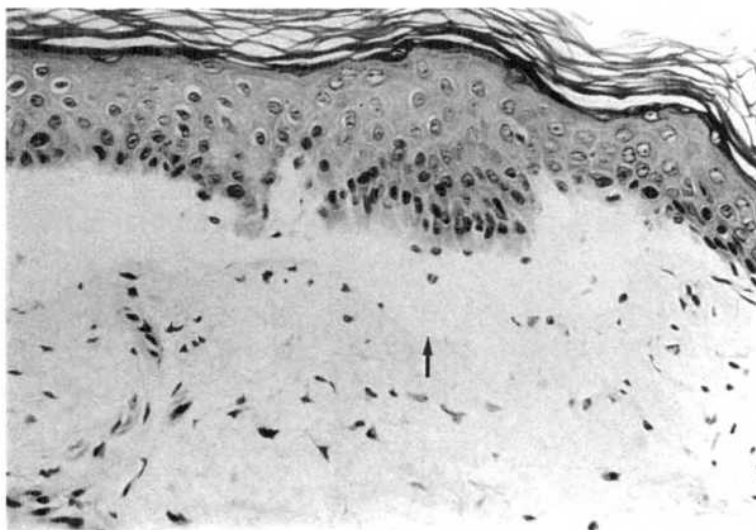


FIGURE 3. Histology shows a sub-epidermal split (arrow) without associated inflammation ($\times 240$).

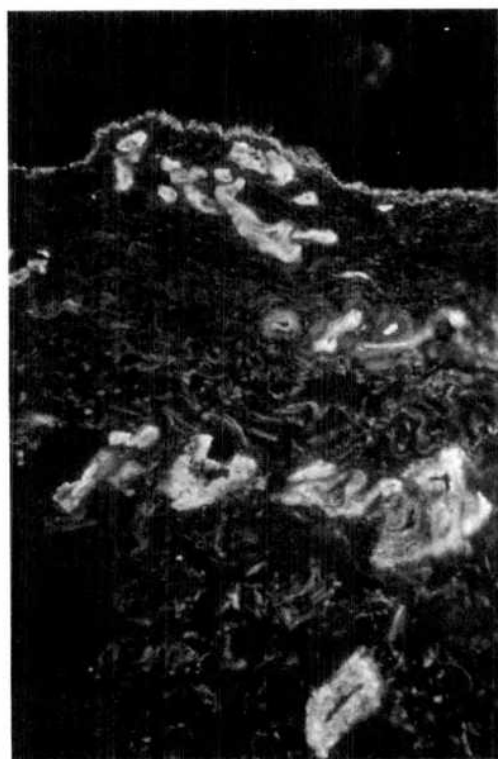


FIGURE 4. Marked perivascular fluorescence with monoclonal antibody to Type IV collagen. The dermo-epidermal basement membrane zone also fluoresces.

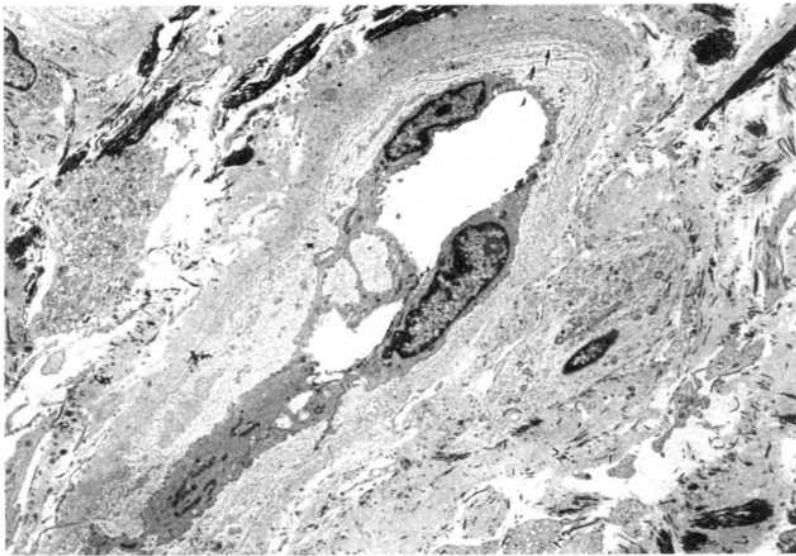


FIGURE 5. Electron micrograph. Marked reduplication of the perivascular basement membrane (arrows) ($\times 3000$).

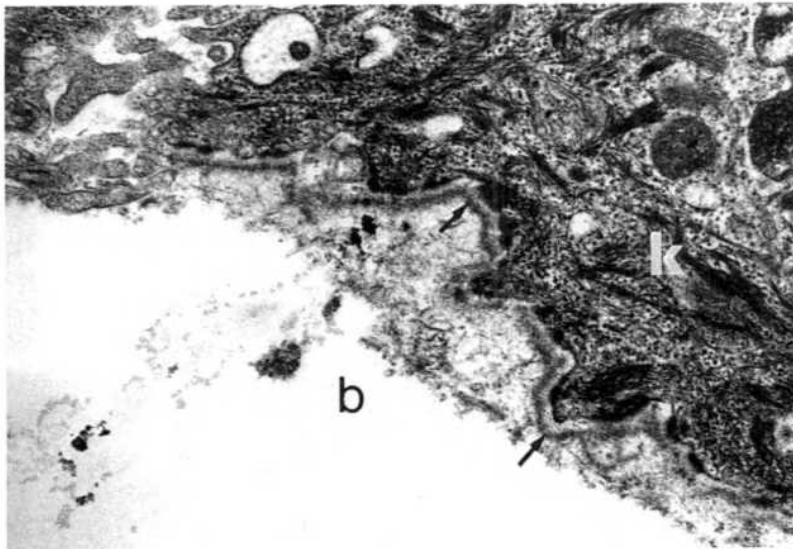


FIGURE 6. Electron micrograph. The blister is sub-epidermal in location (k = keratinocyte, b = blister; Arrows = lamina densa) ($\times 30,000$).

urine, stool and blood, a full blood screen, liver function tests and an autoantibody screen. All these investigations were normal. A skin biopsy was carried out on normal, non-lesional skin and on histology there was some increase in dermal connective tissue cells. No other changes were noted and there was no evidence of amyloid. Direct immunofluorescence was performed and this showed weak granular deposition of IgG at the basement membrane zone, with some

more marked deposition around the upper dermal vessels. Irradiation monochromator tests on the skin were normal.

Case 3

A 47-year-old woman presented with a 3-year history of blistering on the backs of the fingers and dorsa of both hands with occasional milia. She had noted increased skin fragility especially on the dorsa of her hands. The last episode of blistering occurred in March 1988. Medications commenced after this time included ibuprofen and coproxamol. She had taken pyridoxine at the time of onset of lesions, but had not taken this drug for 2 years. She was a regular sun-worshipper and had used a sunbed regularly for 15 years, especially prior to Mediterranean holidays. Since the beginning of 1988 she had stopped using the sunbed.

Examination revealed a moderately tanned individual with no other abnormalities.

Quantitative porphyrin estimations performed on urine, stool, and blood were normal, as were a full blood screen and autoantibody screen.

A skin biopsy of a blister obtained 6 months previously showed a non-inflammatory sub-epidermal blister. Stains for amyloid were negative. Direct immunofluorescence was also negative. Irradiation monochromator tests were normal.

Case 4

A 35-year-old Caucasian female presented with an 18-month history of blisters on the face and backs of the hands, tending to be worse in summer. She had also noted skin fragility, with blisters and erosions occurring after minor trauma. For 3 years she had used a sunbed, spending two 1-h sessions daily for 4 weeks each year. In addition she had sunbathed frequently during 6 months in South America during which time the blistering of the skin developed. Examination revealed scarring at the site of previous blisters on the face and hands. Full blood count and biochemical and autoantibody screening was normal. Quantitative analysis of urine, blood and stool for porphyrins was normal. Direct immunofluorescence of the skin showed a granular pattern of IgM and fibrin at the dermo-epidermal basement membrane zone and perivascular fluorescence with IgG, IgA, C3 and fibrin in the upper dermis. No blisters were present on the skin at the time of investigation. Irradiation monochromator testing was normal. The patient after stopping sunbathing and using a sunbed became asymptomatic. However, with further sunbed exposure she relapsed.

DISCUSSION

These four patients developed striking skin fragility and blistering as a consequence of considerable sun and prolonged sunbed exposure similar to cases previously described.^{1,2} The clinical picture differs somewhat in Case 1 with marked purpura attributable to scratching in addition to skin fragility and blistering. Cases 2, 3 and 4 were less dramatic but Case 3 had not used a sunbed for 6 months prior to presentation and blistering had abated though the skin fragility persisted. No blisters were present at the time of investigation in Cases 2 and 4, but the IMF findings were similar to those of Case 1. Case 3 had a similar histology but negative immunofluorescence, possibly because of the lack of recent sunbed exposure.

The clinical differential diagnoses included cutaneous porphyria, epidermolysis bullosa acquisita (EBA), cutaneous amyloidosis and drug induced pseudoporphyria. Histological, immunofluorescent, electron microscopic and monoclonal antibody studies of type IV collagen showed changes typical of porphyria.^{8,9} However, porphyrin analysis excluded porphyria. The

immunofluorescence pattern was not that of EBA, but more in keeping with that seen in porphyria⁸ or pseudoporphyria.⁵

Acute effects of sunbed exposure include erythema, intense itching, burning sensations and dryness of the skin, freckling, and polymorphic light eruption.¹⁰ Other photodermatoses may be aggravated or precipitated and photosensitizing drugs or chemicals, whether systemic or topically applied may also cause acute adverse effects. Infections such as pityriasis versicolor,^{11,12} common warts¹³ and recurrent herpes simplex have also been reported with sunbed use.

The long-term sequelae of sunbeds are potentially more serious. Studies in hairless mice¹⁴ and humans¹⁵ have shown dermal connective tissue damage and chronic UVA exposure is now linked with epidermal tumour production in mice.^{16,17} Sunbed exposure has been implicated as an aetiological factor in development of melanomas^{18,19} and a recent study indicates that patients who develop melanomas are more likely to have used a sunbed or ultraviolet lamp in the recent past, compared with a matched control population.²⁰ Immunological changes in the skin have been observed in those who have used sunbeds for 12 1/2-hour sessions.^{21,22} Also cataracts may occur with chronic UVA irradiation of the eyes.²³

Fair-skinned individuals are more susceptible to ultraviolet radiation-induced skin changes as a result of chronic sun exposure.²⁴ In this case report the prolonged use of sunbeds was associated with development of cutaneous findings suggestive of porphyria. It is possible that the chromophore responsible for the cutaneous damage in these patients is porphyrin present in normal amounts in the blood and skin and which, following excessive UVA exposure over a prolonged period, could lead to porphyria-like tissue damage. It is now clear that sunbed-induced 'pseudoporphyria' should be added to the already long list of adverse effects of sunbed exposure. This is another reason why the use of sunbeds, especially by people with fair skin, should be actively discouraged.

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Brawny cutaneous induration and granulomatous panniculitis in large cell non-Hodgkin's (T suppressor/cytotoxic cell) lymphoma

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SUMMARY

A 56-year-old lady presented with striking peau d'orange-like, woody, diffuse infiltration of the skin of the upper, inner aspects of both arms and thighs. Initial biopsy specimens showed a granulomatous panniculitis with non-specific lymphohistiocytic infiltration. Clinical deterioration occurred with development of ascites and peripheral oedema; a single axillary node became palpable. Cytology of ascitic fluid, and histology and immunopathology of the axillary node, and of a further skin biopsy, now revealed diagnostic changes of a diffuse large cell lymphocytic lymphoma of T suppressor/cytotoxic type. Granulomatous panniculitis resulting in diffuse cutaneous infiltration may rarely be a presenting sign of malignant lymphoma.

Cutaneous involvement occurs in up to 20% of patients with lymphoma other than mycosis fungoides, and includes not only papules, nodules or plaques as a result of specific lymphomatous infiltration of the skin, but also a number of non-specific symptoms and signs such as pruritus, urticaria, ichthyosis, erythroderma, erythema multiforme, erythema nodosum, and herpes zoster.^{1,2} Diffuse, brawny induration of substantial areas of skin is an unusual cutaneous sign of lymphoma.^{3,4} Although a granulomatous pattern is a well recognized feature of the histology of lymph nodes and internal organs in a proportion of patients with Hodgkin's disease and other malignant lymphomata,^{5–12} the occurrence of cutaneous granulomata in association with lymphoma has been rarely documented.^{3,7,13–17} We report the case of a patient who presented with the highly unusual combination of diffuse peau d'orange-like, brawny infiltration of the skin, in association with an initial histological appearance of a reactive granulomatous panniculitis; changes diagnostic of a large cell non-Hodgkin's lymphoma (T suppressor/cytotoxic cell phenotype) appeared only in later biopsies.

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CASE REPORT

A 56-year-old Caucasian female, under follow-up for hypothyroidism, initially presented to her general physician with a 6-month history of asymptomatic cutaneous nodules on the upper inner aspects of both arms and thighs, with no other physical signs. Screening tests for a presumptive diagnosis of thrombophlebitis migrans associated with internal malignancy were undertaken, but were all negative, including a full blood count and ESR, electrolytes and urea, liver function tests, a chest X-ray, gastroscopy and a bone marrow examination. She was referred for a dermatology opinion when biopsy of an axillary nodule, thought clinically to be a lymph node, demonstrated a granulomatous panniculitis rather than lymphoid tissue.

Examination revealed woody, hard, diffuse infiltration of the skin of the upper inner aspects of both arms and thighs, with surface peau d'orange change (Fig. 1). In addition, a few scattered erythematous or violaceous maculopapular lesions were present on the anterior chest. There was no palpable lymphadenopathy or hepatosplenomegaly, and gynaecological examination was



FIGURE 1. Brawny induration of the skin of the right upper inner thigh, with alteration of surface contour and peau d'orange change.

normal. Biopsy of the woody area on one arm confirmed the previous histological appearance of an extensive granulomatous panniculitis (Figs 2a and b). There was a mixed lymphohistiocytic infiltrate largely confined to the subcutaneous fat, with scattered multinucleate giant cells and areas of fat necrosis; acid-fast bacilli were not seen. The cells showed a morphology that was somewhat variable but without frankly atypical forms. The slides were reviewed by a number of pathologists, and the consensus was that the changes favoured a benign reactive inflammatory

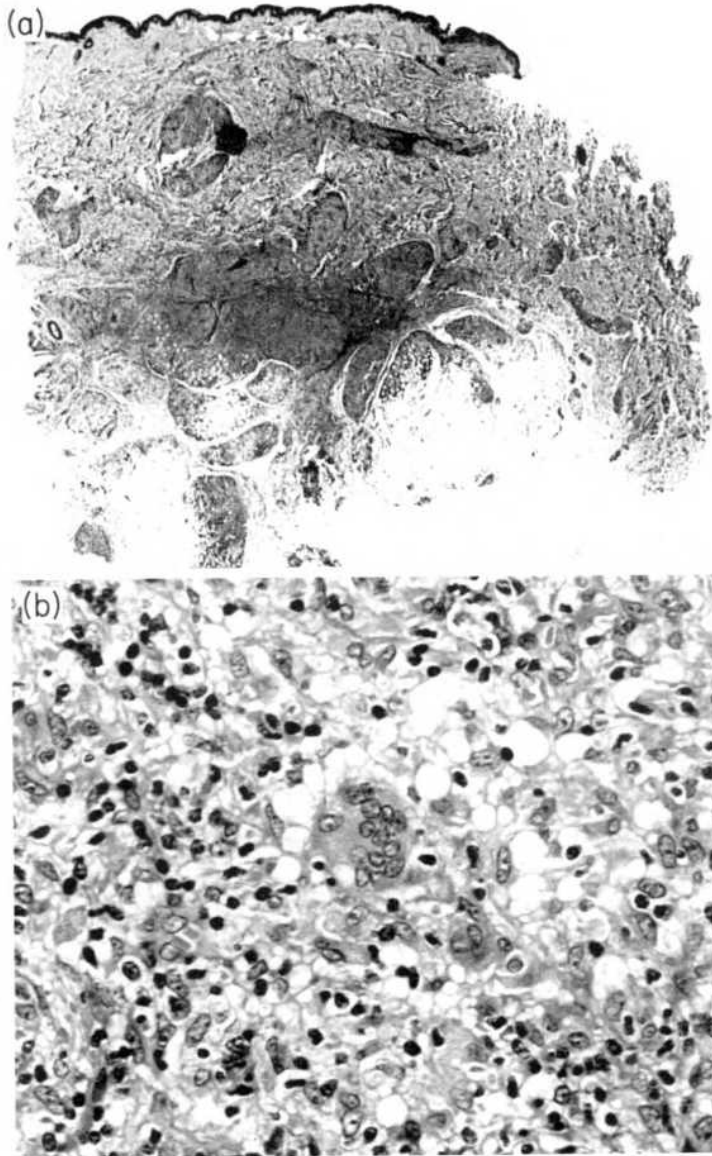


FIGURE 2. (a) Low power view of skin biopsy taken from thigh, showing area of panniculitis. (H & E, original $\times 7.5$). (b) Higher power view showing granulomatous pattern of inflammation in the subcutis, with a mixed lymphohistiocytic infiltrate with multinucleate giant cells. (H & E, original $\times 300$).

process rather than a lymphoma. During the period of investigation, the condition of the patient deteriorated rapidly, with intermittent low-grade fever, malaise, weight loss, mild breathlessness, and development of ascites and dependent oedema.

Investigations

A repeat chest X-ray showed a small right pleural effusion without parenchymal change. A Mantoux test was negative at 1 in 10,000; repeated analyses of sputum and urine, and of pleural fluid aspirate, were negative on direct microscopy, and later on culture were negative for acid-fast bacilli. Liver function became abnormal, with a slightly raised alkaline phosphatase at 310 iu/l (normal < 300), raised aspartate transaminase at 210 iu/l (normal < 55 iu/l), and lowered total protein at 50 g/l (normal > 61 g/l) and albumin at 21 g/l (normal > 35 g/l). Computerized axial tomography of the chest and abdomen demonstrated only the right pleural effusion and the ascites; in particular, no lymphadenopathy was detected.

Histological and immunopathological studies

A liver biopsy showed only extensive steatosis of liver cells and mild central cholestasis, without evidence of lymphomatous infiltrate or granulomatous inflammation. Cytology of ascitic fluid aspirate did, however, show atypical lymphocytes with pleomorphic and clefted nuclei.

A single small lymph node became palpable in the left axilla. Histological examination of this node (Fig. 3a and b), and of a further skin biopsy, now revealed changes diagnostic of a diffuse large cell lymphocytic lymphoma. Immunoperoxidase staining of sections of skin with the monoclonal antibodies UCHT 1 (anti-CD3), UCHT4 (anti-CD4) and UCHT8 (anti-CD8) was performed on frozen tissue according to standard techniques, and showed that the majority of the cells in the infiltrate were of T suppressor/cytotoxic phenotype (Fig. 4a and b).

Treatment and course

The patient was commenced on chemotherapy with vincristine and intermittent courses of cyclophosphamide, adriamycin, methotrexate, etoposide, and prednisolone in various combinations. There was a good initial response, with shrinkage of cutaneous lesions, and decrease in the rapidity with which ascitic fluid reaccumulated following aspiration. Unfortunately, she then succumbed to an episode of acute peritonitis complicated by pneumonia; a post-mortem examination was not obtained.

DISCUSSION

Extensive brawny induration of the skin and subcutis, with resultant surface peau d'orange change, is a very unusual finding in malignant lymphoma, but was the presenting sign in our case. To our knowledge, only two similar cases have previously been reported: Randle *et al.*³ documented the case of a patient with a diffuse large cell lymphoma who presented with oedematous infiltration around one elbow, and Saekow *et al.*⁴ reported brawny induration of the chest wall resembling the 'en cuirasse' appearance of metastatic breast carcinoma in a patient with established diffuse, poorly differentiated lymphocytic lymphoma.

In our patient, the initial histological picture was that of a granulomatous panniculitis with a mixed lymphohistiocytic cell infiltrate. It was not till later that changes diagnostic of a large cell non-Hodgkin's lymphoma appeared in biopsies of skin and lymph node. The occurrence of sarcoidal reactions, with non-caseating epithelioid granulomata, has been well-documented in association with malignant lymphomata affecting internal organs, especially in relation to

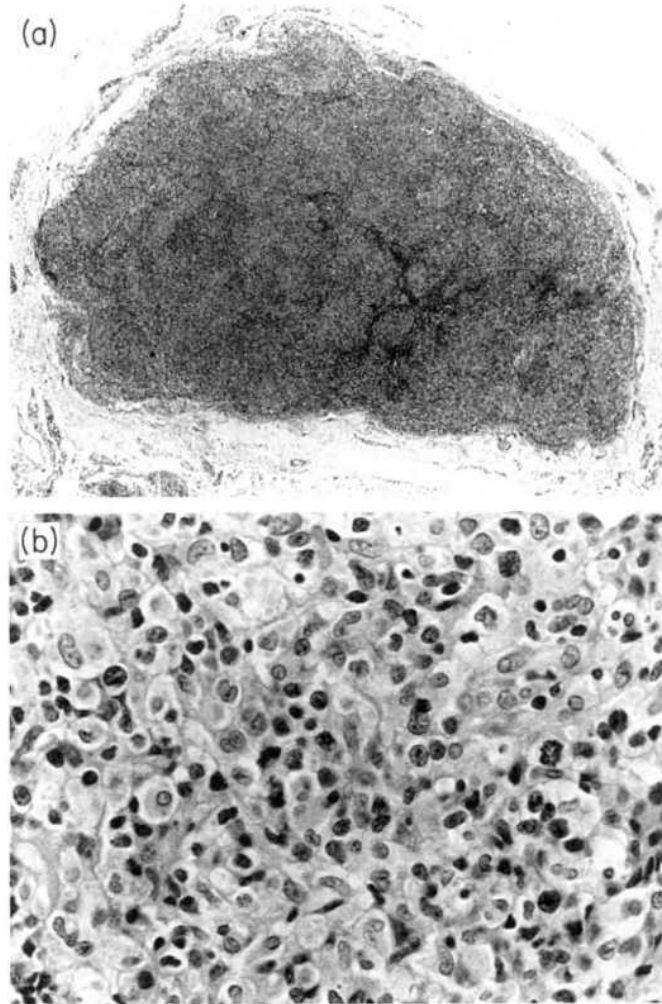


FIGURE 3. (a) Low power view of left axillary lymph node to show architecture disrupted by lymphomatous infiltration. (H & E, original $\times 15$). (b) Higher power view of node to show atypical large lymphocytes with abnormal cleaved nuclei. (H & E, original $\times 390$).

Hodgkin's disease.⁵⁻¹² Cutaneous granulomatous reactions have been reported only rarely in association with lymphoma of the skin, both in relation to mycosis fungoides^{7,13-15} and to other non-Hodgkin's lymphomata.^{3,16,17} In our patient, immunohistochemical staining identified the majority of the infiltrating cells as of T suppressor/cytotoxic cell phenotype. As in other reported cases,^{3,12,17} the finding of a granulomatous histology in early biopsies from our patient obscured the neoplastic nature of her condition, and delayed the final diagnosis of malignant lymphoma. Re-examination of the earlier biopsies once the diagnosis became clear did not allow a definitive diagnosis of lymphoma to be made on the basis of the skin changes. Unfortunately, T-cell receptor gene rearrangement studies were not carried out to determine whether at this stage a minority malignant clone was present. Nevertheless, since atypical cells were seen in the

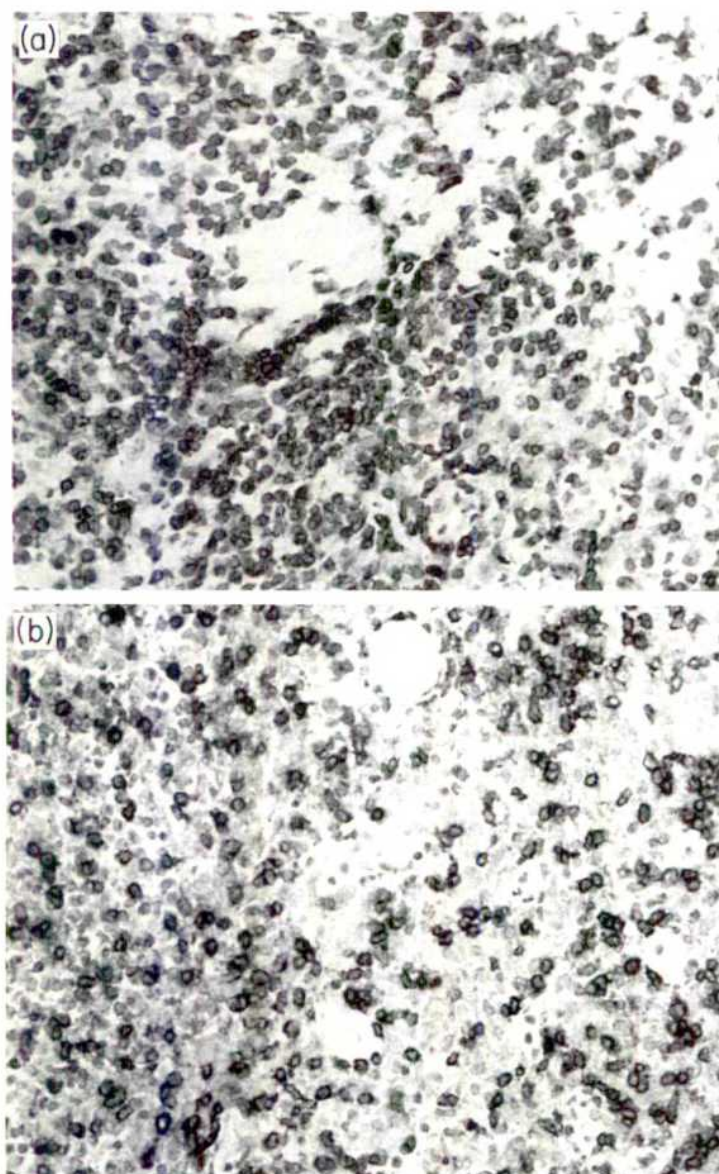


FIGURE 4. Immunohistochemical staining of skin biopsy with monoclonal antibodies. (a) UCHT 1 (pan T cell marker). (original $\times 225$). (b) UCHT 8 (suppressor/cytotoxic T cell subset). (original $\times 225$).

later skin biopsy taken from an area of woody induration, it would seem likely that the clinical appearances and granulomatous panniculitis in our case were the result of a lymphoma in the skin rather than of a granulomatous reaction to a lymphoma at a distant site. Although it is exceptionally rare as a cause of granulomatous panniculitis, our case illustrates that lymphoma should be included in the differential diagnosis, especially when the clinical presentation is atypical.

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Nail dystrophy in cutaneous T-cell lymphoma

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SUMMARY

A case is reported of cutaneous T-cell lymphoma in an elderly woman. Dystrophy of all 20 nails was caused by a lymphomatous infiltrate of the nail apparatus.

Cutaneous T-cell lymphoma and Sézary syndrome may involve the nails.¹ Zaias states that the histopathology of the nail lesions in mycosis fungoides is identical to that of skin.² This case presents the histological and immunocytochemical findings from a patient with adult T-cell lymphoma presenting with cutaneous features.

CASE REPORT

An 84-year-old woman presented with a 7-month history of a pruritic rash, diffuse alopecia, malaise and weight loss. Two months previously she had developed a painful nail dystrophy with marked functional disability. Examination revealed a widespread eczematous papular rash over the trunk and limbs. Similar changes were present in the skin over the dorsum of the interphalangeal joints. Erythema and scaling of the finger pulps were prominent. There was a diffuse, non-scarring alopecia with erythema of the scalp.

All 20 nails were dystrophic. Marked subungual hyperkeratosis separated the nail plate from the nail bed producing a picture similar to that seen in pachyonychia congenita (Fig. 1). The nail plates were roughened with loss of the normal sheen.

Small firm lymph nodes were present in the groins and the liver was palpable 2 cm below the costal margin.

Investigations

Haematological investigation revealed a white cell count of 17.7×10^9 with 57% lymphocytes, some of which were noted to have nuclear clefting. Immunocytochemical typing of circulating lymphocytes showed positive staining with an anti-CD5 monoclonal antibody Tu71, a pan T-cell marker which is found in lymphoproliferative states and usually stains Sézary cells,³ but was

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FIGURE 1. Dystrophic, roughened nails with marked subungual hyperkeratosis.



FIGURE 2. Nail matrix showing epidermal acanthosis, hyperkeratosis and a dense dermal lymphocytic infiltrate.

negative for the anti-CD7 monoclonal antibody Tu93 which detects peripheral T-cells, particularly after activation, but rarely stains Sézary cells.⁴

Biopsy from lesional skin on the trunk showed a band-like infiltrate of mononuclear cells in the papillary and subpapillary dermis. Large atypical cells with hyperchromatic nuclei were evident. Within the epidermis individual lymphocytes and small Pautrier microabscesses were present. Immunocytochemical analysis showed the abnormal cells to be T-helper cells with the larger ones staining for the CD30 (Ki-1) antigen. This antigen detects a small population of rapidly proliferating large cells and is present in conditions such as lymphomatoid papulosis, mycosis fungoides, Hodgkin's disease and some large cell non-Hodgkins lymphomas.⁵

Longitudinal biopsy of the left fourth finger nail showed similar histological features. The nail plate was separated from the nail bed by a mass of eosinophilic debris. There was acanthosis of the epidermis around the proximal nail fold which continued into the matrix and nail bed. Below this was a dense infiltrate of mononuclear cells (Fig. 2) which, at higher power could be seen within the epidermis (Fig. 3). The phenotype of the infiltrating cells was similar to that seen in the skin biopsy exhibiting considerable pleomorphism and dysplasia with abnormal mitotic forms. Immunocytochemical staining showed the infiltrate to be CD5 and CD30 positive as had been found in the skin. PAS preparation showed no evidence of fungus in the nail.

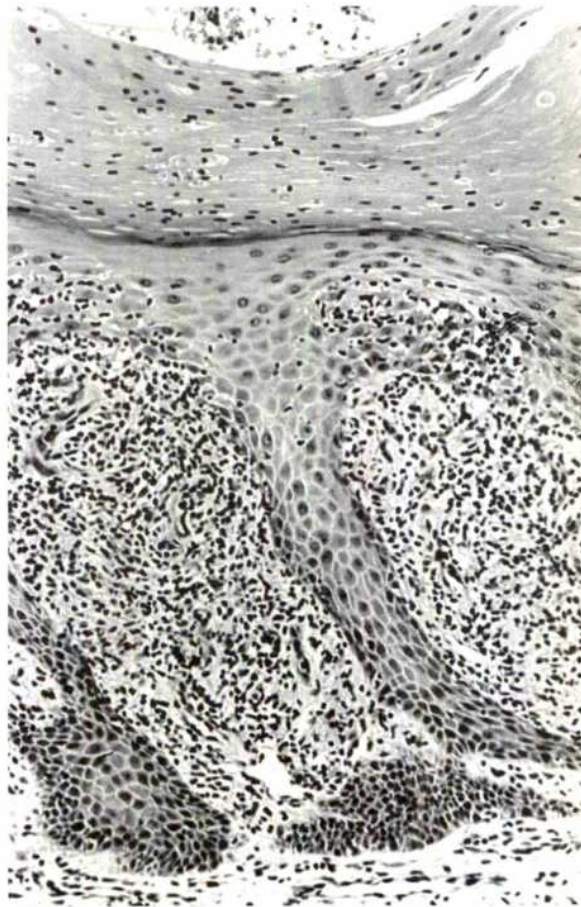


FIGURE 3. Aggregates of lymphocytes within the epidermis of the nail matrix.

Progress

The skin lesions responded partially to topical steroids but the patient was unable to manage her treatment. Prednisolone (30 mg daily) was, therefore, substituted. The rash improved but the patient developed nausea and vomiting due to malignant hypercalcaemia. This failed to respond to saline infusion and was treated with a diphosphonate. Bone marrow aspirate and trephine revealed lymphomatous infiltration. Low dose chlorambucil therapy was introduced. The skin lesions improved over the next month but the nail dystrophy remained unchanged. Chlorambucil therapy was stopped due to a falling white cell count. The patient developed bronchopneumonia and died at home.

Discussion

Nail dystrophy is an unusual feature of cutaneous T-cell lymphoma. Although Zaias has reported nail bed hypertrophy in mycosis fungoides,² the histological changes have not been illustrated previously. Nail dystrophy similar to that described in this patient has been reported in association with palmo-plantar hyperkeratosis in a case of mycosis fungoides.⁶ The nails returned to normal during remission after chemotherapy. Histology of the nails was not performed. Sonnex *et al.*⁷ described nail changes in five patients with Sézary syndrome. They suggested that these changes were either due to a non-specific effect of chronic erythroderma or due to T-cell infiltration of the nail unit. The histological findings in this patient indicate that the nail dystrophy is caused by the same pathological process which occurs in the skin.

ACKNOWLEDGMENTS

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Kaposi-like acroangiodermatitis in an above-knee amputation stump

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SUMMARY

The clinical, histological and immunohistological features of a Kaposi-like acroangiodermatitis in an above-knee amputation stump are reported. There was no evidence for an underlying arteriovenous malformation and the irregular purple-blue skin lesions resulted from chronic circulatory disturbance of the distal stump caused by a poorly fitting suction-type prosthesis.

Kaposi-like acroangiodermatitis or pseudo-Kaposi's sarcoma is a reactive angiodysplasia of the cutaneous blood vessels that resembles true Kaposi's sarcoma, both clinically and histologically.^{1,2} The disorder is usually confined to the distal extremities, especially the toes and was described in association with congenital arteriovenous malformations,^{2–6} acquired vascular fistulae,^{7–9} and chronic venous insufficiency.¹⁰ In 1957, Lawton *et al.*¹¹ reported similar lesions in the stump skin of two patients with leg amputations and with post-traumatic vascular fistulae. We recently observed another case of Kaposi-like acroangiodermatitis in a patient with an above-knee amputation. In this patient, the development of the angiodysplasia was induced by a poorly fitting prosthesis.

CASE REPORT

A 31-year-old, overweight man was referred to us in 1986 for diagnosis and treatment of Kaposi's sarcoma that involved the amputated leg. The left thigh and upper left arm were amputated in 1974 as a result of a motor-cycle accident. The amputated stump of the leg was fitted with a suction-socket type prosthesis. In 1983, the patient was fitted with a new artificial limb of the same type that had little or no contact with the distal amputation stump. Over the following years a number of painful purple-blue skin lesions developed in the stump.

Physical examination revealed several purple-blue to brown plaques and papules on the

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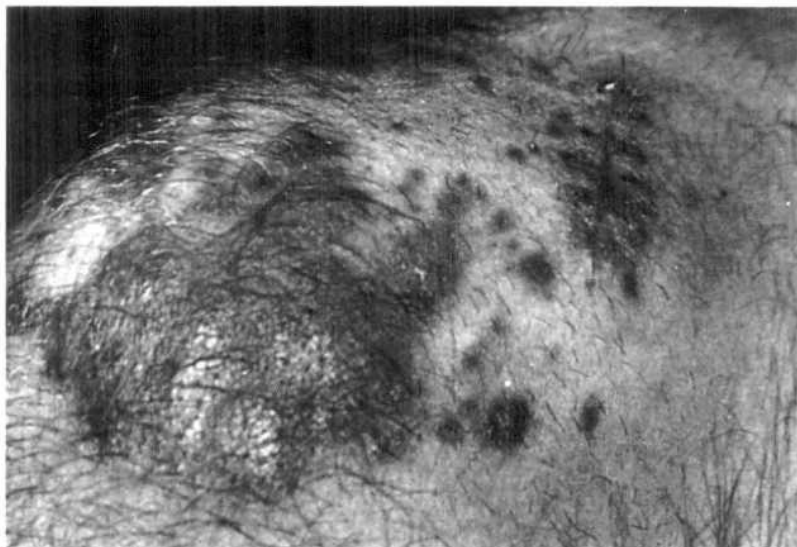


FIGURE 1. Amputation stump showing plaques and papules on the medial aspect of the thigh.



FIGURE 2. Atrophic scar on the lateral side of amputation stump.

medial aspect of the distal part of the amputation stump (Fig. 1). The irregularly shaped lesions were somewhat lichenified and scaly. On the lateral side of the thigh, there was atrophy of the skin and soft tissue and a large traumatic amputation scar (Fig. 2). The end of the stump was swollen and cooler than the upper thigh. There were no skin lesions on the stump of the left arm which had no prosthetic device.

No antibodies were found against human immunodeficiency virus (HIV) using the standardized enzyme-linked immunoabsorbance assay.

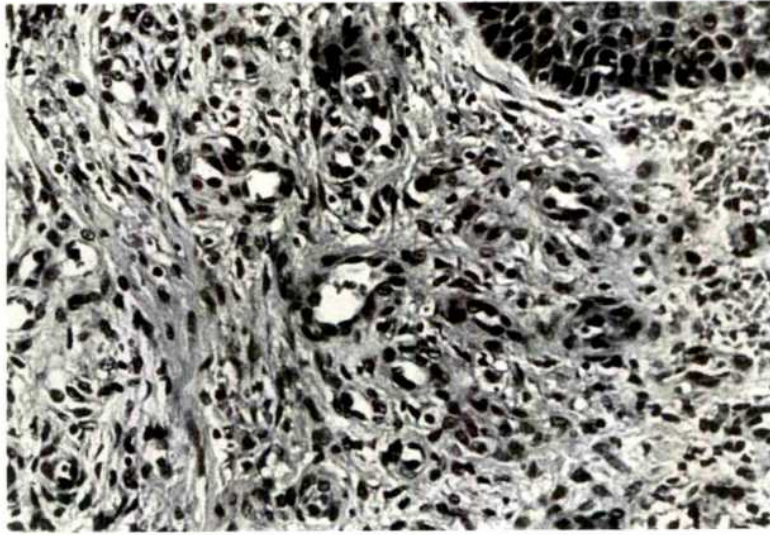


FIGURE 3. Histopathology of the Kaposi-like tumours. Numerous blood vessels with pleomorphic endothelial cells and extravasated erythrocytes (haematoxylin-eosin, $\times 150$).



FIGURE 4. Immunohistological staining with the proliferation associated antibody Ki67. The dermal capillaries show only few positive endothelial cells (arrows). The epidermis displays strong labelling of the basal and suprabasal keratinocytes (haemalum counterstaining, $\times 80$).



Biopsies of the skin tumours showed, on histology, acanthosis of the epidermis with hyperorthokeratosis. In the upper dermis there were numerous vascular spaces with extravasated erythrocytes, prominent haemosiderin deposits and a moderate increase of fibroblasts (Fig. 3). The vascular channels were lined by pleomorphic endothelial cells, but no atypical cells or vascular slits were observed. A similar proliferation of small to medium sized venous formations was seen in close proximity to the follicular structures of the middle and lower dermis. There was a sparse mononuclear inflammatory cell infiltrate in the dermis. On immunohistology, the vessels stained strongly with an anti-factor VIII antibody (Dianova, Hamburg, F.R.G.). Less than 5% of the endothelial cells (Fig. 4) reacted with the monoclonal antibody Ki67 (Dianova, Hamburg, F.R.G.), indicating that most cells were in the G0 phase of the cell cycle.¹² The blood vessels showed a positive reaction with the monoclonal antibody EN 7/44 (C. Sorg, Münster, F.R.G.). This antibody is specific for endothelial proliferations as seen in angiogenesis and does not react with normal vessels in the skin.¹³

Clinical and radiological examination showed that the lateral side of the amputated leg stump was insufficiently padded by muscle and soft tissue (Fig. 5). The end of the bone was adherent to the overlying amputation scar. A percutaneous femoral arteriogram revealed numerous vascular collaterals in the stump but there was no evidence of arteriovenous fistulae.

Surgical revision of the amputation stump was carried out and a new prosthesis fitted. Fifteen



FIGURE 5. X-ray of the amputated thigh. Note the insufficient soft tissue on the lateral and distal part of the stump (arrows).

months after the operation, the patient had an amputation stump that gave him no trouble and was without any dermatological or orthopaedic problems.

DISCUSSION

Many skin problems can affect amputation stumps of limbs,¹⁴ including: circulatory disturbances with hyperaemia; oedema; stasis dermatitis; haemangiomas; lymphangiomas and occasionally lymphangiosarcomas (Stewart-Treves syndrome). Our patient showed angioma-tous skin lesions that clinically resembled Kaposi's sarcoma. There were multiple purple-blue to brown plaques and papules in the distal part of the amputation stump. No regional or widespread lymph-node swelling was observed and there was no serological evidence for an HIV infection.

Definite diagnosis of Kaposi-like acroangiokeratitis was made by the histological and immunohistological features of the tumours. On histology, the lesions were characterized by a marked proliferation of small blood vessels and fibroblasts, and with extravasated erythrocytes and deposits of haemosiderin in the dermis. In contrast to true Kaposi's sarcoma,¹⁵⁻¹⁷ no vascular slits and no atypia of the nuclei of endothelial cells were observed. The low mitotic activity of the pleomorphic endothelial cells was confirmed with immunohistological staining. The proliferation associated antigen Ki67¹² was expressed by less than 5% of the nuclei of the cells. On the other hand, the blood vessels showed a positive reaction with the monoclonal antibody EN 7/44. This antibody stains proliferating and migrating endothelial cells of reactive angiomas.¹³

Most cases of pseudo-Kaposi's sarcoma that have been reported are associated with an underlying arteriovenous malformation, being either congenital or traumatically acquired.²⁻⁹ The haemodynamic shunts can sometimes be identified by palpable thrill and by a bruit, and also by an increased warmth of the involved area of skin.¹⁸⁻²⁰ The histology often reveals abnormal communications between the cutaneous arterioles and venules.²⁰ Proof of the arteriovenous malformations is usually revealed by percutaneous arteriography.^{11,20}

The reactive angiodysplasia on the amputated leg described by Lawton *et al.*¹¹ was due to traumatically acquired vascular fistulae. In our patient, by contrast, there was no clinical, histological, or arteriographic evidence for underlying arteriovenous anastomoses. Remarkably, the lesions developed 9 years following a leg amputation and after the patient had a poorly fitting prosthesis that was only in partial contact with the distal stump. The pressure on the distal stump was unevenly distributed and this disturbed the circulation and led to oedema. These alterations of the circulation may have resulted in a reactive proliferation of small blood vessels and fibroblasts as has been suggested for the development of Kaposi-like acroangiokeratitis as seen in chronic venous insufficiency.^{4,10} To our knowledge, the present case report is the first description of a reactive angiodysplasia induced by a poorly fitting limb prosthesis.

ACKNOWLEDGMENT

We thank Professor C. Sorg for providing the monoclonal antibody EN 7/44.

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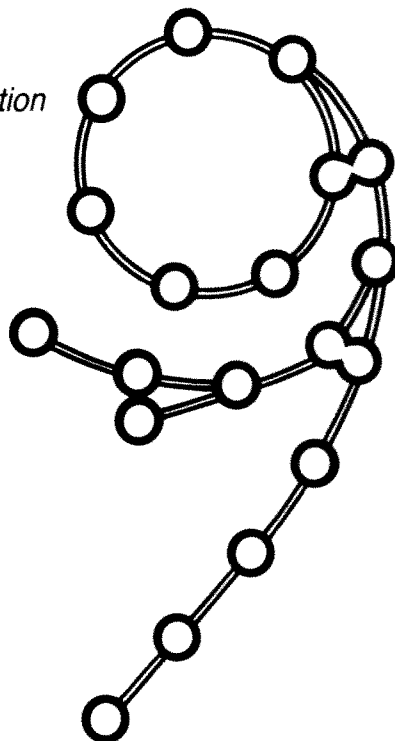
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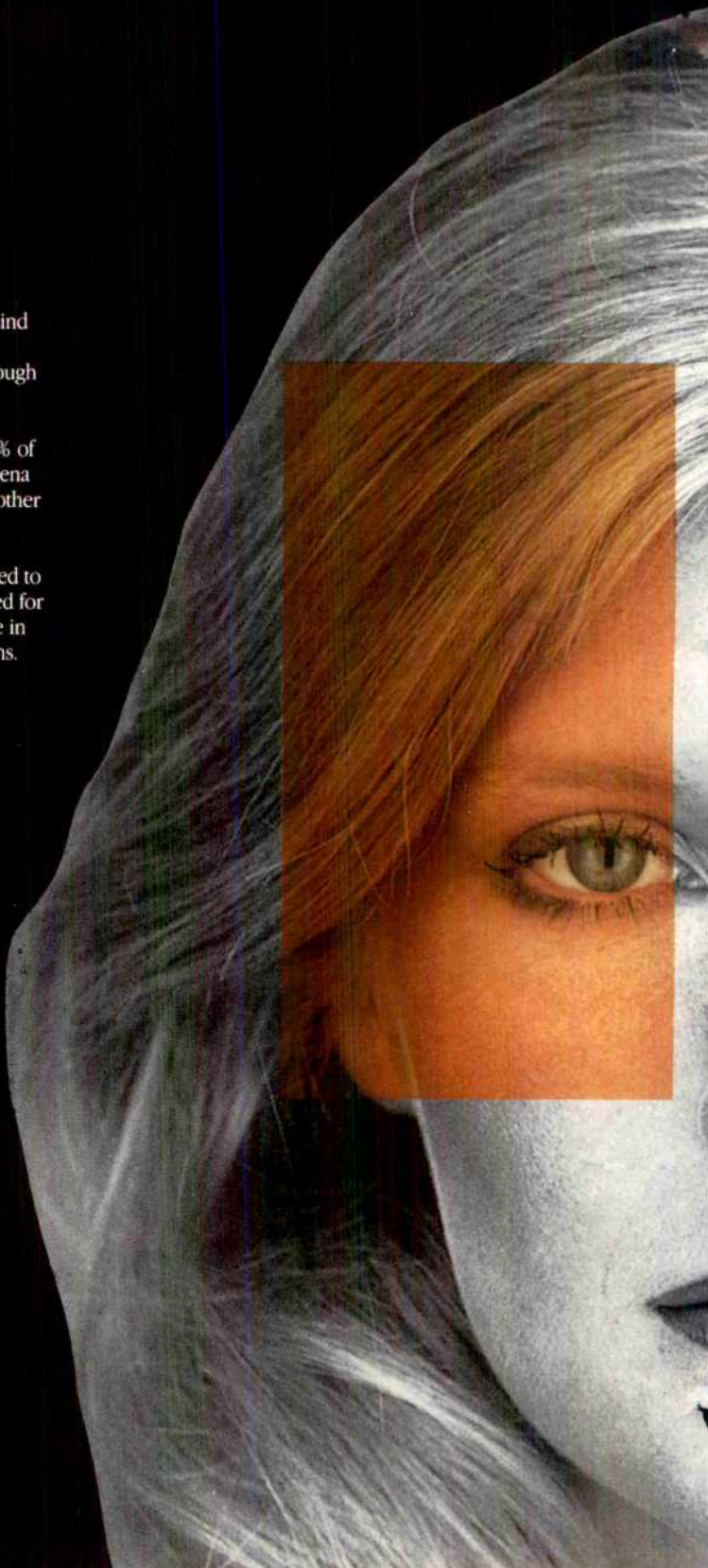
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Topical eicosapentaenoic acid (EPA) in the treatment of psoriasis

SIR, There has been considerable interest recently in the efficacy of fish oil in some inflammatory diseases. Fish oil contains eicosapentaenoic and docosahexaenoic fatty acids which are metabolized along similar pathways as arachidonic acid but give rise to less active products (with the exception of prostacyclin¹). Several studies^{2,3} have reported the beneficial effects of fish oil (MaxEPA® capsules) when administered orally to patients with psoriasis. Bjorneboe *et al.*⁴ found no benefit giving EPA to patients with psoriasis⁴ but did observe an improvement in those with atopic eczema⁵ as was also found with a topical preparation.⁶

We wish to report the results of a small pilot study using MaxEPA 10% in Unguentum Merck® and compared with the vehicle alone. The study was a single-blind within-patient comparison of 7 weeks duration, which had local ethical committee approval. The psoriasis was scored for severity of scaling, infiltration and erythema on a 0-6 point scale. Patients applied active or placebo creams twice daily to selected lesions of psoriasis that were of similar size and severity and kept diary cards to record the subjective symptom scores. The amounts of creams used were determined by weighing the returned containers.

Of the 11 patients studied, eight showed clinical improvement of EPA-treated lesions compared with placebo. The results are outlined in Table 1, which shows mean scores for severity and the standard error.

TABLE 1. EPA treated lesions compared with placebo

Lesion	MaxEPA-treated		Placebo-treated	
	Erythema	Scaling	Erythema	Scaling
Admission	2.7 ± 0.09	2.14 ± 0.12	2.57 ± 0.07	2.14 ± 0.09
Week 7	2.14 ± 0.14	1.43 ± 0.10	2.71 ± 0.13	1.71 ± 0.13

Differences between treatments at the end of the study were significant at the 5% level (Mann-Whitney *U*-test). The diary card data supported the clinical assessments and all but two of the patients considered that the active treatment was superior to placebo. Topical treatment is usually preferable to systemic and the patients considered the transient smell of the cream to be more acceptable than swallowing 10 to 18 large capsules of fish oil daily. We are presently engaged in further studies to evaluate this treatment.

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Drug interactions with methotrexate

SIR, Drug interactions with low dose methotrexate appear to be increasing. There was one case of bone marrow failure due to methotrexate toxicity reported between 1980 and 1983 and eight between 1984 and 1987, of which two were fatal. Four of these interactions were associated with cotrimoxazole,¹⁻⁴ three with non-steroidal anti-inflammatory drugs (NSAIDs),⁵⁻⁷ one with trimethoprim combined with a NSAID⁸ and one with penicillin combined with frusemide.⁹ The following case illustrates the potential seriousness of drug interactions with methotrexate.

A 70-year-old woman with a 40-year history of chronic plaque psoriasis had been treated with 5 mg of oral methotrexate once weekly for 7 years. In July 1987 she was treated for 7 days with oral cotrimoxazole 960 mg b.d. for acute bronchitis while continuing treatment with methotrexate. Seven days after finishing cotrimoxazole she presented with spontaneous bruising and a petechial rash on the ankles. Her haemoglobin was 13.7 g/l, mean cell volume was 88.8 fl (87-89), total white cell count was $3.5 \times 10^9/l$ (4-10) and platelet count was $17 \times 10^9/l$ (150-400) with a bleeding time of 9 min. Bone marrow trephine biopsy showed a hypocellular marrow with virtually no megakaryocytes. Methotrexate was stopped, the last dose being 3 days previously. After 5 days the white cell and platelet counts had risen to $7.4 \times 10^9/l$ and $185 \times 10^9/l$ respectively with no treatment. The most probable explanation for methotrexate toxicity in this case is the combined antifolate effects of methotrexate, sulphamethoxazole and trimethoprim. It is unlikely that displacement of methotrexate from binding sites on plasma proteins would significantly increase free methotrexate levels. This is because plasma protein binding of methotrexate is less than 70%, and the drug is distributed quickly into total body water and so relatively little remains in plasma.¹⁰ However, we cannot exclude the possibility that renal tubular excretion of methotrexate was reduced by cotrimoxazole.

Bone marrow failure due to drug interactions with methotrexate is most likely to result from profound inhibition of dihydrofolate reductase and consequently severe intracellular folate depletion. This raises two points. Firstly, daily folic acid supplementation probably ameliorates the intracellular folate depletion that occurs during long-term once weekly methotrexate and might, therefore, reduce the risk of methotrexate toxicity or its severity should it occur.^{11,12} We have found that daily folic acid supplements do not appear to reduce the efficacy of methotrexate in psoriasis. This idea should be explored further. Secondly, since the risk of serious drug interactions with methotrexate seems to be considerable, we feel that all patients should be provided with written and verbal information specifically warning about this.

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Fixed drug eruptions: 77 cases from 1981 to 1985

SIR, The fixed drug eruption (FDE) is a cutaneous reaction in which there are one or several erythematous plaques or blisters that are fixed in the same place and provoked by a drug. The eruption fades, to leave slate-brown macules. Upon challenge with the causative agent the FDE reappears at the same site and new lesions also may appear. The drugs most frequently associated with FDE have been barbiturates, phenazone derivatives and less frequently tetracyclines, sulphonamides and acetosalicylic acid.¹⁻³

We have previously described a 10-year series of patients with FDE.³ The comparison of this previous series with the present one which has been carried out over the period from 1981 to 1985 shows that phenazone derivatives are the most important causative agents of FDE, whereas the number of FDEs caused by barbiturates has diminished (Table 1). The drop in the incidence of barbiturate-induced FDE

TABLE 1. Drugs responsible for fixed drug eruption
1971-1980 and 1981-1985

Drug	No. of cases	
	1971-1980	1981-1985
Phenazones	36	32
Barbiturates	22	6
Sulphonamides	8	8
Tetracyclines	5	5
Chlormezanone	5	1
Trimethoprim	2	3
Carbamazepine	—	3
Other or unknown	8	19
Total	86	77

from 25% to under 10% is almost certainly due to the decreased use of barbiturates. In the present series 14 of the cases were of severe generalized type of FDE and the causative drugs were phenazone derivatives in nine of the patients.

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Psoriasis: improvement during and worsening after pregnancy

SIR, Knowledge about the potential effects of pregnancy on psoriasis is of importance to many patients, but information given to patients is often anecdotal. We have carried out a questionnaire study to determine whether psoriasis is indeed affected by pregnancy.

Questionnaires were sent to all 282 female patients aged 18–38 years with psoriasis who first attended the Dermatology Department, University Hospital of Wales between January, 1983 and June, 1988. The questionnaires asked for simple information about whether the patients had been pregnant since the onset of their psoriasis and how their psoriasis had been affected during these pregnancies. This study was carried out with the approval of the Ethical Committee of the University Hospital of Wales.

Out of the 282 patients questioned, 151 (53.5%) replied. Of these, 65 (43.0%) patients had had one or more pregnancies since the diagnosis of their psoriasis and 69 (45.7%) had had no pregnancies. 17 (11.3%) blank questionnaires were returned. Of the 65 women who had had a successful pregnancy since their psoriasis started, 34 (52.3%) had one pregnancy, 15 (23.0%) had two pregnancies and 16 (24.6%) had three or more pregnancies, a total of 118 pregnancies. The average interval between the diagnosis of psoriasis and first pregnancy for the 65 patients in the sample was 8.5 years ($SD \pm 6.1$) (median 7 years).

In the 65 women who had been pregnant since the start of their psoriasis, 27 (41.5%) reported no change in their psoriasis during pregnancy. However 25 (38.5%) women reported improvement, and only eight (12.3%) reported worsening of their psoriasis (χ^2 -test = 7.7, one degree of freedom, continuity correction applied, $P = 0.005$). Inconsistent effects were noted in 5 (7.7%) patients with more than one pregnancy.

In the 112 pregnancies in whom information was given, there was no change in psoriasis in 48 pregnancies (42.9%), improvement in 46 (41.1%) and worsening in 16 (14.3%). In two pregnancies the patient 'did not know'. The patients were diagnosed with psoriasis for the first time during their first pregnancy. Two patients remarked that their psoriasis had always been unstable before, during and after gestation. Of those patients who noticed a change in their psoriasis during pregnancy this usually occurred at the end of the first trimester or in the second trimester.

During the 3 months post partum of these 65 women, 18 (27.7%) reported no change in their psoriasis. However, only seven (10.8%) women reported an improvement and 35 (53.8%) reported worsening of their psoriasis (χ^2 -test = 17.4 $P < 0.001$). In four patients with more than one pregnancy, inconsistent effects were noted and one patient 'did not know'. In the 112 pregnancies in whom information was given, there was no change of psoriasis in the post partum period in 41 (36.6%), an improvement in 12 (10.7%) pregnancies, and a worsening in 55 (49.1%). In four pregnancies the patient 'did not know'.

This study suggests that during pregnancy, if psoriasis alters it is more likely to improve than worsen. On the other hand if psoriasis changes in the post partum period, it is much more likely to deteriorate than improve. The changes during pregnancy are closely consistent with those reported by Farber and Nall;¹ in patients with psoriasis 32% improved during pregnancy and 18% 'worsened', but information on post partum changes was not given. The alteration in psoriasis during and after pregnancy may be related to the modulation of the immune system during pregnancy.²

These findings have clinical relevance as they draw attention for general practitioners and dermatologists to prospectively make advice on treatment available for psoriasis patients following delivery.

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ACKNOWLEDGMENT

We wish to thank Dr T.Peters for statistical advice.

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Book Reviews

Pediatric Dermatology. Edited by L.A.SCHACHNER AND R.C.HANSEN. (1988). New York: Churchill Livingstone. 2 volumes Pp. 1641. Price £225.

This book is the first-ever attempt to put together a definitive textbook of paediatric dermatology. It is a testimony to the success of North America's Society for Pediatric Dermatology, which has now reached its thirteenth year of existence, and whose members have collaborated in its production. There are 52 contributing authors, all but one of whom work in the US. The presentation of the book is immaculate. The text is well organized and beautifully laid out. Most of the illustrations are of good quality, but as is usual where colour transparencies have been converted into black and white prints, some fail to show what they are supposed to show. It is a pity that the trouble was not taken to replace or remove these. It seems to me to be generally the case that the quality of clinical photographs in modern dermatology texts is poorer than it was in the past, and everyone involved in the production of books should reflect on this change for the worse. As is often the case in books on paediatric dermatology, some of the photographs are of adults, which really should not happen in a serious book like this one. The colour plates are grouped at the beginning of each volume; again, this should not happen; they should be dispersed in the right places in the text despite the cost.

I must confess that, overall, the book was a real disappointment to me. Of course, one could argue that it was not written for full-time paediatric dermatologists. The quality of the individual chapters varies considerably; this is in some ways inevitable in a multi-author work. However, it seemed to me that the writing of some of the chapters had been delegated to junior colleagues and it showed, in a less-than-authoritative result. For a book of this size, designed to be comprehensive, I found the amount of information on individual conditions often disappointingly sparse, and sometimes frankly inadequate. Extraordinarily brief consideration is, for example, given to the ectodermal dysplasias. One would at least expect literature references to be given where discussion in the text is very abbreviated; a rare but important condition like lipoid proteinosis is dealt with in just a few words without any references being provided in compensation. I looked up vulvovaginitis, a not-uncommon problem in the prepubertal child. The text said nothing about the common variety seen in children and managed, albeit unwittingly, to imply that the problem was one of sexual abuse. Such an approach suggested that the authors of this chapter had not given sufficient thought to the required paediatric orientation. Several other authors have had the same problem; I was amazed to find topical testosterone even mentioned as a possible therapy for genital lichen sclerosus in female children.

Lack of space is not a good excuse for the brevity of the discussion of some important disorders, because in other places, space has been wasted as conditions have been considered in more than one chapter. Examples include hypomelanosis of Ito, focal dermal hypoplasia, the chondrodysplasia punctata syndromes, the ichthyoses and aplasia cutis congenita; worse still, in none of these cases are the other contributions cross-referenced.

I must add that there is much of real quality in this book. Nevertheless, the high price and beautiful appearance had led me to expect something better. Of course, such a book represents an extraordinary amount of work on the part of the editors and the authors, and one should, therefore, hesitate to be too critical, at least of the first edition. There is a real chance, next time round, of turning this book into the outstanding reference work it should be.

D.J.ATHERTON

Dermatology in Five Continents. Edited by C.E. ORFANOS, R. STADLER AND H. GOLLNICK. (1988). Proceedings of the 17th World Congress of Dermatology held in West Berlin in May 1987. Heidelberg: Springer-Verlag.

Over 6,500 dermatologists attended this congress and the editors must be congratulated in reducing the proceedings to a manageable, single volume.

All aspects of the congress are reported, from the opening ceremony to the closing remarks. The book is then divided, as was the congress, into eight separate sections: special lectures, seminars for continuing education, advances in dermatology, symposia, workshops, courses, posters and short communications.

The special lectures are fully covered and give an overview of some rapidly changing fields of research, such as growth factor biology. The seminars and advances sections contain good short reviews, with references, of a wide range of subjects, perhaps with a slight over-emphasis on the problems of AIDS. The reporting on the symposia and workshops is varied, some subjects, notably paediatric dermatology has a full and detailed account of what took place, other subjects have only a brief summary of the days events. One or two of the reports on the courses, notably that on photodermatology, is almost a small text book within the book. The poster section is a collection of abstracts and the short communications are reported as title and author only.

With over 1000 contributors to this volume it really is a large collection of abstracts. The book would be a useful addition to a library as a source of a quick overview, by a recognized expert, of a subject and its contemporary references.

S.I. WHITE

Books Received

Handbook of Leprosy. W.H. JOPLING AND A.C. MCDUGALL (1988) 4th Edition. Heinemann Professional Publishing.

A Guide to Leprosy Control. World Health Organization (1988) 2nd Edition.

Essays on Leprosy. Edited by T.J. RYAN AND A.C. MCDUGALL Published for St. Francis Leprosy Guild by Slade Hospital, Department of Dermatology Oxford: Alden Press.

WHO Expert Committee on Leprosy. Technical Report Series 768, 6th Report (1988).

News and Notices

British Society for Investigative Dermatology: Annual Meeting

The next Annual Meeting of the British Society for Investigative Dermatology will be held at the University of Birmingham on 21–22 September 1989.

Further information and abstract forms are available from Dr P.S. Friedmann, c/o British Association of Dermatologists, 3 St. Andrew's Place, London NW1 4LB. The closing date for receipt of completed abstract forms is the 16 June 1989.

Fourth Annual Symposium for Dermatology Clinical Assistants

The fourth annual symposium for Dermatology Clinical Assistants will be held at the Holiday Inn in Leicester, U.K. on 9–11 June 1989. The symposium, which is sponsored by Kirby-Warrick Pharmaceuticals, will cover advances in dermatological surgery, skin and psyche, and diseases of the genitalia. There will be workshop sessions on leg ulcers, occupational skin disease, ageing and the skin and the skin and systemic disease.

All clinical assistants should receive an invitation in due course, but if you are in any doubt, please contact: Dr Robin Graham-Brown, Department of Dermatology, Leicester Royal Infirmary, Leicester LE1 5WW, U.K.

International Hair Symposium, 7–8 April, 1989, Dallas, Texas

The first Baylor International Hair Symposium will take place in Dallas on 7–8 April 1989. The topics covered will include the basic science of hair growth, histopathology, endocrine and pharmacologic effects and disorders of hair.

For further information contact Betsy Jennings, Baylor Hair Research and Treatment Center, 3600 Gaston Avenue, Ste. 1057, Dallas, Texas 75246 U.S.A.

John Milne Memorial Fund

Applications are invited for travelling scholarship(s) funded by the John Milne Memorial Fund. These are for travel to dermatological meetings or to centres which the applicant wishes to visit to increase his/her knowledge, understanding and experience either of clinical problems or of research techniques. The scholarships are open to clinicians and scientists for travel during 1989 and the total value will not exceed £500.

Applications, including a full curriculum vitae and reasons for travel, should be sent to Professor R.M. MacKie, University of Glasgow, Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU, by 30 April 1989.

XIth Course on Pediatric Dermatology, Arcachon, France, 28 March–1 April, 1989

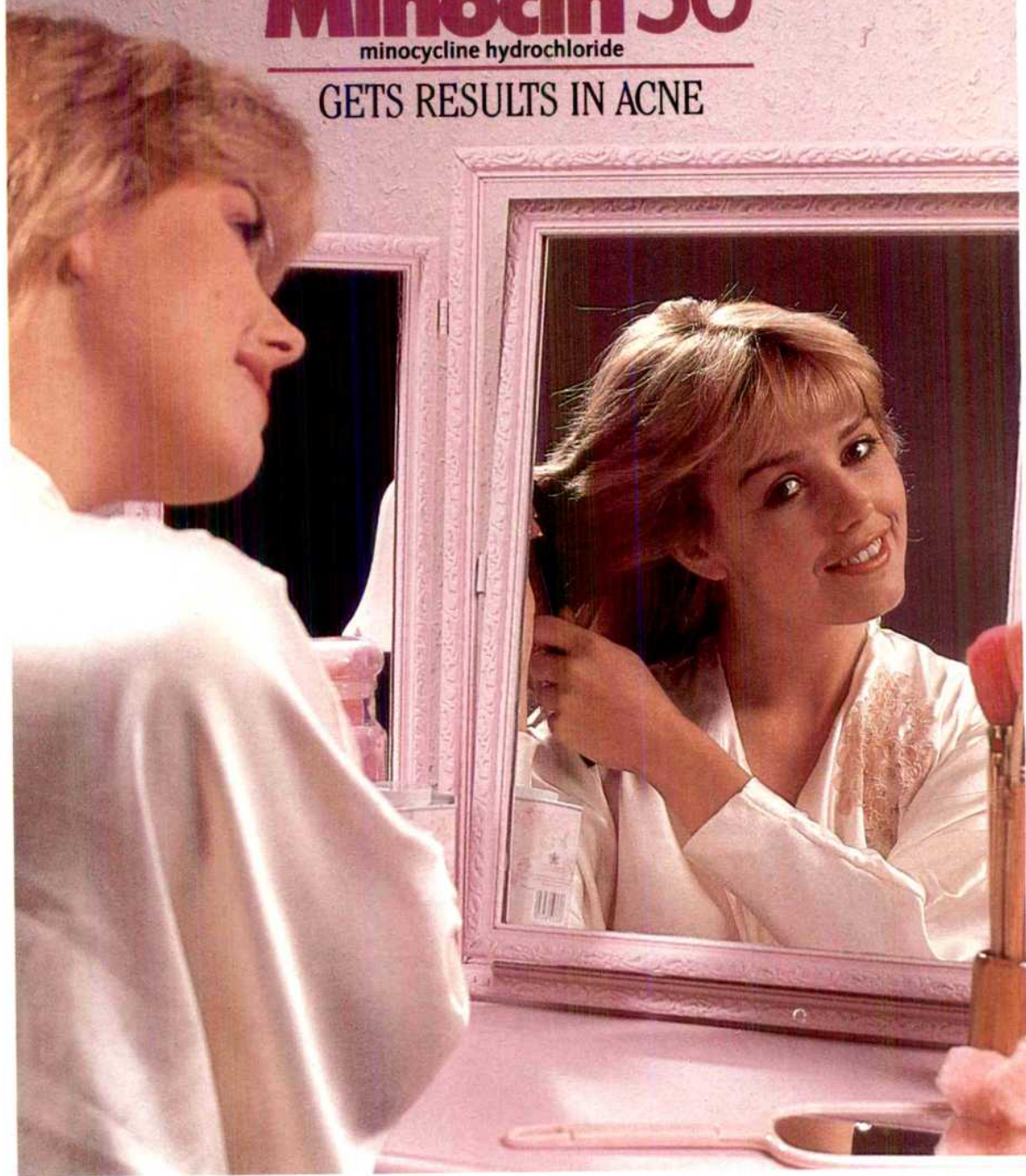
The XIth course on Pediatric Dermatology will be held in Arcachon (near Bordeaux) from 28 March to 1 April, 1989. The course will be given in French and the topics covered will include congenital abnormalities, genital disorders, inflammatory and immuno-allergic conditions and toxic eruptions of childhood. For further information contact: Professor J. Maleville, Service de Dermatologie Hopital des Enfants, 168, cours de l'Argonne, F. 33077 Bordeaux Cedex, France.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts. Three copies of all material for publication should be sent to the Editor, Professor S. S. Bleehen, Dermatology Department, Royal Hallamshire Hospital, Sheffield S10 2JF. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	<i>g</i>
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometre(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

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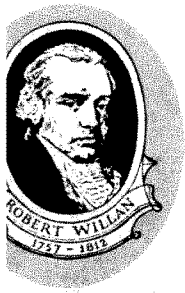
British Journal of Dermatology, Volume 120, Number 4, April 1989

CONTENTS

PAGE

Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation S.LISBY, E.RALFKIAER, R.ROTHLEIN AND G.L.VELSGAARD	479
A flow cytometric study of Langerhans cell histiocytosis JANET MCLELLAND, JULIA A.NEWTON, MARIAN MALONE, R.S.CAMPLEJOHN AND A.C.CHU	485
Suppression of contact sensitivity by local hyperthermia treatment due to reduced Langerhans cell population in mice A.YOSHIOKA, Y.MIYACHI, S.IMAMURA, M.HIRAOKA, S.JO AND M.ABE	493
Triiodothyronine alters the cornification of cultured human keratinocytes R.R.ISSEROFF, K.T.CHUN AND R.M.ROSENBERG	503
An assessment of the value of Ag NOR staining in the identification of dysplastic and other borderline melanocytic naevi RONA M.MACKIE, S.I.WHITE, MORAG M.SEYWRIGHT AND H.YOUNG	511
The normal range in diagnostic phototesting B.L.DIFFEY AND P.M.FARR	517
Inhibition of proliferation of HeLa cells by dithranol (anthralin) and 10-butyryl dithranol (butantrone) ANITA REMITZ, ARJA-LEENA KARINIEMI, EERO LEHTONEN AND STIG NORDLING	525
Identification of the epidermolysis bullosa acquisita antigen by LH 7-2 monoclonal antibody: use in diagnosis F.M.TATNALL, P.C.WHITEHEAD, M.M.BLACK, F.WOJNAROWSKA AND I.M.LEIGH	533
The pathogenesis of lichen planus A.GILHAR, T.PILLAR, G.WINTERSTEIN AND A.ETZIONI	541
Pityriasis rotunda as a cutaneous marker of hepatocellular carcinoma: a comparison with its prevalence in other diseases I.BERKOWITZ, H.J.HODKINSON, M.C.KEW AND A.M.DIBISCEGLIE	545
Epidermal dysplasia and cyclosporine therapy in renal transplant patients: a comparison with azathioprine D.SHUTTLEWORTH, R.MARKS, P.J.A.GRIFFIN AND J.R.SALAMAN	551
Sunbed-induced pseudoporphyria G.M.MURPHY, J.WRIGHT, D.S.H.NICHOLLS, P.H.MCKEE, A.G.MESSENGER, J.L.M.HAWK AND G.M.LEVENE	555
Brawny cutaneous induration and granulomatous panniculitis in large cell non-Hodgkin's (T suppressor/cytotoxic cell) lymphoma J.ASHWORTH, A.T.COADY, R.GUY AND S.M.BREATHNACH	563
Nail dystrophy in cutaneous T-cell lymphoma K.DALZIEL, N.R.TELFER AND R.P.R.DAWBER	571
Kaposi-like acroangioidermatitis in an above-knee amputation stump G.KOLDE, J.WÖRHEIDE, R.BAUMGARTNER AND EVA-B. BRÖCKER	575
Correspondence	
Topical eicosapentaenoic acid (EPA) in the treatment of psoriasis CATHERINE E.DEWSBURY, PAMELA GRAHAM AND C.R.DARLEY	581
Drug interactions with methotrexate B.J.LIDDLE AND J.R.MARSDEN	582
Fixed drug eruptions: 77 cases from 1981 to 1985 SAKARI STUBB, KRISTIINA ALANKO AND SAKARI REITAMO	583
Psoriasis: improvement during and worsening after pregnancy S.F.DUNNA AND A.Y.FINLAY	584
Book Reviews	585
News and Notices	587





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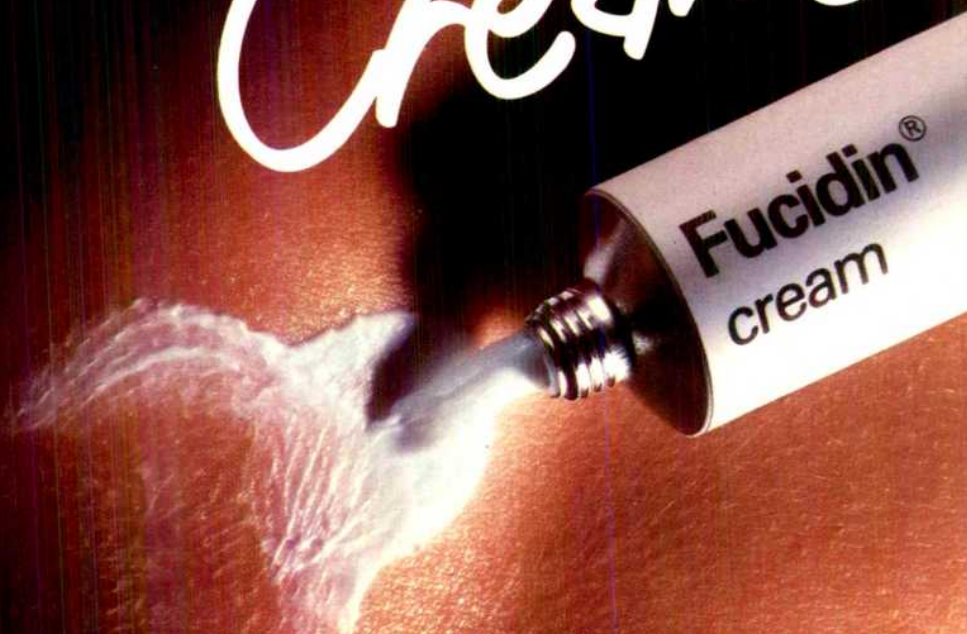
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sticky and irritant.

Prescribing Information Indications: Skin infections caused by sensitive Staphylococci, Streptococci and Corynebacteria. Dosage and Administration: Apply 3-4 times daily to uncovered lesions. Contra-indications, warnings, etc: Contra-indicated in infections caused by non-susceptible organisms and patients with hypersensitivity to fusidic acid and its salts. Caution in pregnancy and lactation. As with all topical antibiotics bacterial resistance may occur. Caution when used near the eye. Side-effects: Hypersensitivity reactions have been reported rarely. Product Licence Number: 0043/0065. Basic N.H.S. Price (15 g): £2.67. Active Ingredients: 2% fusidic acid.



Further information available from: Leo Laboratories Limited, Longwick Road, Princes Risborough, Aylesbury, Bucks HP17 9RR.
*Registered Trade Mark.



It's starting to turn heads

topical **Stiemycin®**

Part of growing up. 2% erythromycin

The first
erythromycin
solution
for inflammatory
acne

- At least as effective as oral tetracycline¹
- Early and sustained effect¹
- Well tolerated in short and long term use^{2,3,4}

PRESCRIBING INFORMATION Presentation: A clear colourless solution in an amber glass screw capped applicator bottle. The applicator allows the product to be applied directly to the involved skin. Each ml contains 20 mg. Erythromycin Base EP in an amount equivalent to 20 mg of Erythromycin base 1st International Standard, in an ethanol base also containing propylene glycol and Laureth 4. **Uses:** Stiemycin is indicated for use in the topical treatment of acne vulgaris. **Dosage and Administration:** To be applied to the affected area twice daily after washing with soap and water. **Contraindications, Warnings and Precautions:** Stiemycin is contraindicated in patients with known sensitivity to any of the ingredients. Concomitant topical acne therapy should be used with caution because a cumulative irritant effect may occur. The use of systemic erythromycin can reduce the excretion rate of warfarin potentiating its effect. The use of Stiemycin in patients who are receiving warfarin is not recommended. Avoid contact with eyes and other mucous membranes. This product is for external use only. **Pharmaceutical Precautions:** Store in a cool place. **Legal Category:** POM. **Package Quantity:** 50 ml. **Basic NHS Price:** £9.00. **Product Licence No.:** PL 0174/0047

- References** 1. Rapaport M. et al. *Cutis* **30** 122-135 (1982).
2. Bernstein MD. et al. *J. Am. Acad. Dermatol.* **4** 318-321 (1980).
3. Eady EA. et al. *J. Am. Acad. Dermatol.* **4** 55-56 (1981).
4. Rivkin I. Rapaport M. *Cutis* **25** 552-55 (1980).



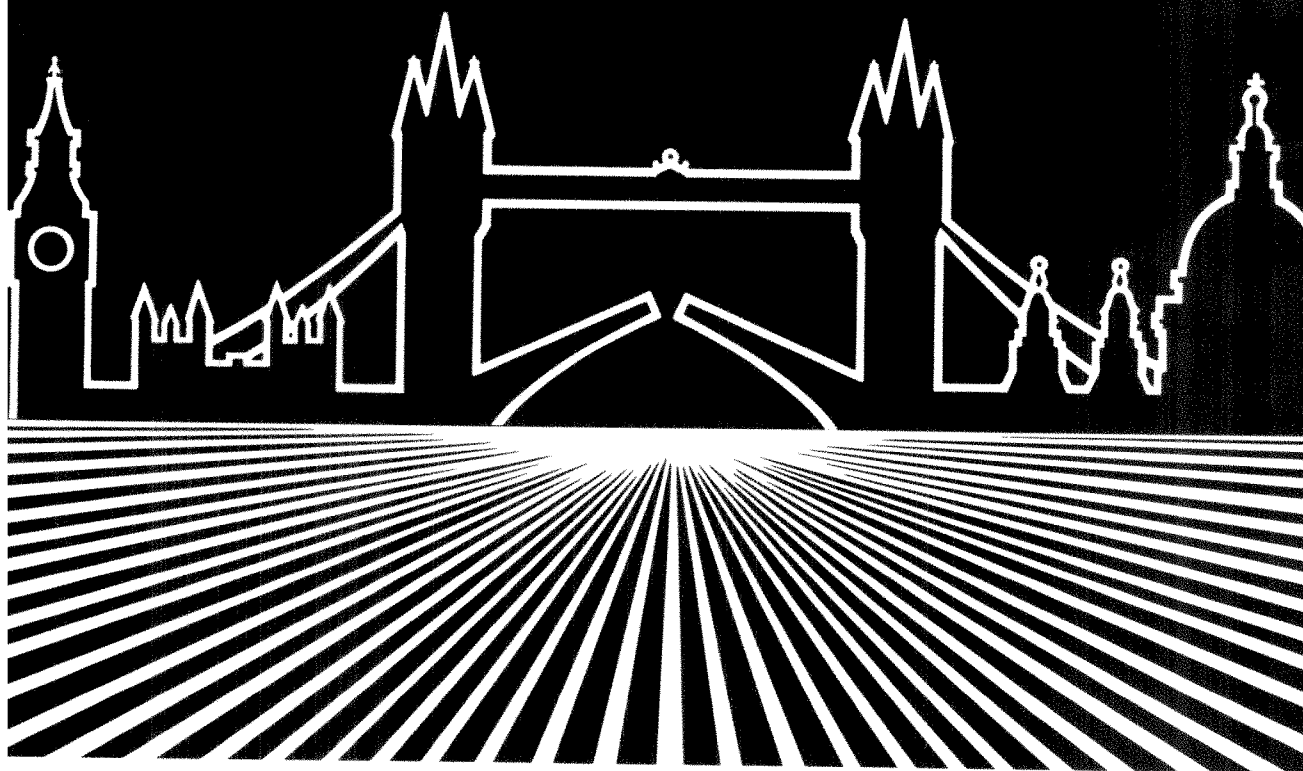
Further information and data sheet available on request from Stiefel Laboratories (UK) Ltd, Hottspur Lane, Wooburn Green, High Wycombe, Bucks. HP10 0AU.



An International Symposium

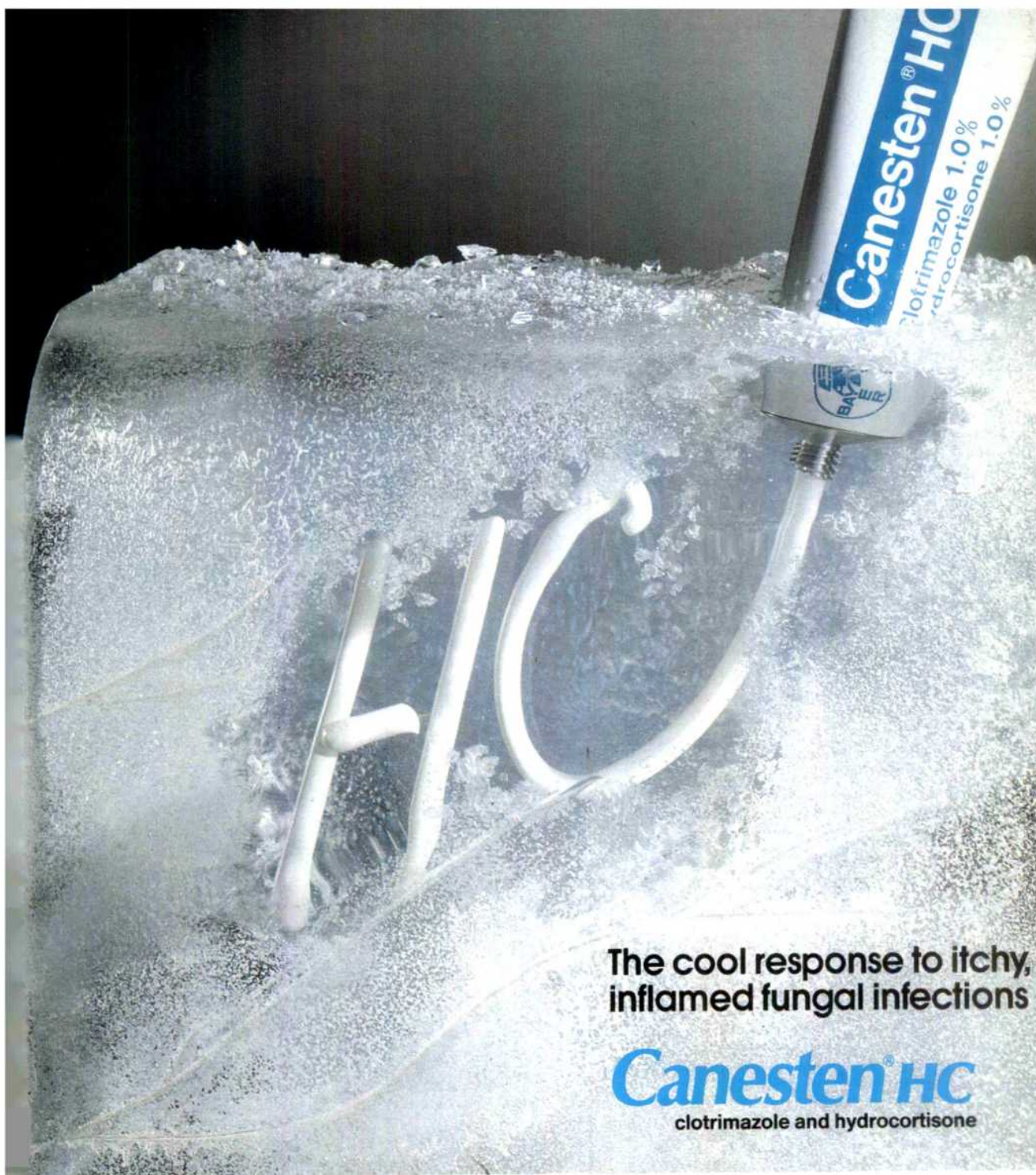
Clinical Dermatology in the year

2000



Further information is available from Professor Malcolm Greaves
Institute of Dermatology St Thomas' Hospital London SE1 7EH, U.K.





The cool response to itchy,
inflamed fungal infections

Canesten® HC
clotrimazole and hydrocortisone

DESCRIPTION INFORMATION Presentation Canesten HC is available as a white cream containing 1.0% Clotrimazole BP and 1.0% Hydrocortisone PhEur. **Uses** The treatment of skin infections due to dermatophytes, yeasts, moulds and other fungi, where co-existing symptoms of inflammation e.g. itching, require rapid relief. **Dosage and Administration** Canesten HC should be thinly and evenly applied to the affected area twice daily and rubbed in gently. **Contra-indications** Hypersensitivity to any of the ingredients. **Warnings and Precautions** As with all corticosteroids, long term continuous therapy to extensive areas of skin should be avoided, particularly in infants and children. In infants, the skin may act as an occlusive dressing and increase absorption. **Side effects** Rarely patients may experience local mild burning or irritation immediately after applying the cream. Very rarely, the patient may find this irritation intolerable and stop treatment. Hypersensitivity reaction may occur. **Use in pregnancy** Topical administration of corticosteroids to pregnant animals can cause abnormalities of foetal development. The relevance of this to humans has not been established. In animal studies clotrimazole has been associated with teratogenic effects but following oral administration of high doses to rats there was evidence of foetotoxicity. The relevance of this effect to topical administration in humans is not known. However, clotrimazole has been used in pregnant women for over a decade without attributable adverse effects. It is therefore recommended that Canesten HC should be used in pregnancy only when considered necessary by a clinician. **Accidental oral ingestion** In the event, routine measures such as gastric lavage should be performed as soon as possible after ingestion. **Pharmaceutical Information** Store in a cool place. **Legal Category** POM. **Basic NHS Price** £3.18 per 30 g. **Product Licence Number** PL0010/0120.

Baypharm 

Further information available from:
Bayer UK Limited,
Pharmaceutical Business Group,
Bayer House, Strawberry Hill,
Newbury, Berkshire RG13 1JA.

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(University of London)

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13 - 17th. November 1989

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All major problems will be covered, including: Eczematous, psoriasiform and lichenoid reactions; vasculitis and panniculitis; metabolic skin diseases; infectious and non-infectious granulomas; viral dermatoses, vesiculo-bullous diseases; epidermal, adnexal and mesenchymal tumours; melanocyte and naevocytic malformations and tumours; benign and malignant lymphoid infiltrates.

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Course fee (inc. catering): £275

Application forms from:

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London W12 0NN Telephone: 01 740 3117

THE UNIVERSITY OF MICHIGAN DEPARTMENT OF DERMATOLOGY

is seeking an outstanding dermatologist to join the Clinical Dermatopharmacology Unit. The position represents an exceptional opportunity for dermatologists to begin or expand an academic career. The clinical research involves the development and testing of new dermatologicals, most of which are at the forefront of dermatopharmacology. A staff is in place to assist with the research. Direct performance of research (all of which involves patients or normal volunteers) is expected, as is supervision of residents involved in clinical research. No previous experience in clinical research is required as substantial opportunity for on-job learning is available. The position involves participation in all aspects of clinical research in dermatology including new drug development, protocol development, monitoring of studies, clinical evaluation of research subjects, and statistical analysis. A wealth of previously and concurrently collected data is available for development into publications. Percentage of time devoted to laboratory investigations is negotiable. Candidates should have completed their general dermatologic training and should be interested in a career in academic dermatology, the pharmaceutical industry, or both.

The opportunity is a fellowship position until the applicant can obtain full licensure to practice medicine; subsequently, the successful physician may apply for a full-time faculty position. The position is currently available.

Ann Arbor provides excellent working and living conditions. The department is one of the largest in the United States and is part of one of the premiere university medical schools.

Interested physicians should send their curriculum vitae to Charles N. Ellis, M.D., Director, Dermatopharmacology Unit, University of Michigan Medical Center, Department of Dermatology, 1910 Taubman Center, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109-0314, U.S.A.

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Fungal nail problem?

Trosyl Nail Solution contains an antifungal agent in a penetrating base, specifically formulated to overcome the problem of permeating nail tissue.

Trosyl Nail Solution has been shown to produce an improvement in over two thirds of patients with fungal nail infection.

Additionally, the combination of Trosyl Nail Solution with griseofulvin has been shown to substantially increase cure rates compared to griseofulvin alone.

ticloconazole
TROSYL*
nail solution

For further information,
call Freephone Pfizer.

Prescribing Information.

Indications: Nail infections due to susceptible fungi (dermatophytes and yeasts). Dosage: Apply every twelve hours for up to six months, or twelve months, if necessary. Contra-indications: Hypersensitivity to imidazoles. Pregnancy. Side-effects: Local irritation. Package quantities and Basic NHS Cost: Bottles of 12ml containing ticloconazole 28% w/w, £25.00 (PL57/0236). Full information on request.



Pfizer Limited, Sandwich, Kent.

*Trade Mark 21146

THE HARD FACTS OF LIFE

One family in seven is being brought up by a lone parent
40% of all one-parent families are headed by a divorced mother
One in five children will see their parents divorce
In 1987, 23% of all births were outside marriage

Behind the statistics are parents who have to carry the burden of responsibility, on their own. Whatever the cause - death, divorce, separation or abandonment - being a lone parent is a difficult task. Doing the work of two parents is hard enough, but all too often, these families live on half the income of a two-parent family. They are more likely to suffer from poverty, poor housing, unemployment and inadequate childcare facilities.

We understand the problems lone parents face and the important role that caring professionals have in supporting them. We offer free advice to lone parents and their advisers and provide a wide range of information on single-parent families. Through campaigning we have gained important rights for one-parent families. But much more still needs to be done.

By making a donation, you will be helping us to continue our vital work. If you make a covenant you can increase your gift by one third, at no extra cost to you. Please help to give the children in these families a brighter future.

For further information contact: Anne Fitzgerald, National Council for One Parent Families, FREEPOST, 255 Kentish Town Road, London NW5 1YA, tel. 01-267 1361.

Kracht is soms vereist. Veiligheid altijd.

Strikt genomen gelden voor een scheepshuid dezelfde wetmatigheden als voor de huid van de mens: onderhoud is essentieel en zelfs dan zijn er wel eens aandoeningen waartegen krachtig moet worden opgetreden. Alleen is de mens niet van staal en kan een grofmazige behandeling soms meer kwaad dan goed doen. Preferid is een uniek preparaat om de menselijke huid te helpen bij de behandeling van ernstige eczematuze aandoeningen en psoriasis. Preferid is een sterkwerkend, niet gefluoreerd topicaal corticosteroid met een geringe systemische invloed. Preferid heeft bovendien een snel intredend effect. Op grond van de snelle biotransformatie is het risico van systemische invloed relatief gering. Preferid. Uniek in de combinatie van kracht en geringe systemische invloed.



Preferid

De nieuwe generatie sterkwerkende topicale corticosteroiden

Indicatie: Per gram crème/zalf: 0,25 mg budesonide. **Indicaties:** Psoriasis en ernstige of hardnekkige huidaandoeningen met een eczematuze genese. **Contra-indicaties:** Overgevoeligheid, bacteriële-, virale-, parasitaire- en gistinfecties, ulceruze huidaandoeningen. **Bijwerkingen:** Bij langdurige behandeling kan remming van de bijnierschorsfunctie optreden. Lokale bijwerkingen zijn: atrofie, periorale dermatitis, teleangiëctasieën. **Waarschuwing:** Niet op de oogleden toepassen. **Presentatievormen:**

Gist-brocades
FARMA BV
Postbus 1000 7300 KB Heteren/HK

A NEW BRITISH PHARMACEUTICAL COMPANY

LEADERS IN RESEARCHING ESSENTIAL FATTY ACIDS

Scotia Pharmaceuticals, the newly formed ethical pharmaceutical division of Efamol Holdings PLC, is a world leader in the field of Essential Fatty Acids (EFAs).



As a research-based organisation, completely independent of any other pharmaceutical company, Scotia Pharmaceuticals is committed to developing original products in its own laboratories.

Scotia Pharmaceuticals' scientists are actively researching new, naturally-derived sources of EFA's and investigating their application to treating a diversity of disorders.

A MAJOR CONTRIBUTOR TO
FURTHERING THE UNDERSTANDING
OF ABNORMAL BIOCHEMISTRY



Scotia Pharmaceuticals is making a substantial contribution to the elucidation of biochemical mechanisms in disease. The knowledge gained, particularly in the field of lipid research, is being applied to devising unique approaches to therapy.

Scotia's innovative therapeutic concepts, based on naturally-occurring sources, are already proving to be of significant importance in the development of new pharmaceutical products.

Scotia Pharmaceuticals is currently involved with original research into a number of areas including skin diseases such as atopic eczema and seborrhoeic dermatitis; complications of diabetes; rheumatoid arthritis; menstrual disorders and certain cancers.

A close-up photograph of a single, vibrant yellow flower. The flower has five large, slightly ruffled petals and a central cluster of numerous stamens with long, thin filaments. The background is dark and out of focus, showing some green foliage.

Essential Fatty Acids or EFAs are naturally-occurring substances which cannot be synthesised by the body and must therefore be obtained from the diet. They are essential components of the structure of all cell membranes and are precursors of many short-lived molecules which regulate cell function.¹² The main dietary EFA, linoleic acid, has to be converted to gamma-linolenic acid (gamma-linolenic acid – GLA) to be fully useful to the body.



A REVOLUTIONARY ORAL
APPROACH TO ATOPIC ECZEMA

Scotia Pharmaceuticals has been instrumental in demonstrating that many patients with atopic eczema are unable to form adequate amounts of GLA from dietary linoleic acid.³ GLA and its metabolites are especially important in the maintenance of healthy skin.^{4,5}

Easy-to-swallow EPOGAM capsules simply contain a selected variety of Evening Primrose Oil, a naturally occurring source of Essential Fatty Acids, which is rich in GLA.

Studies performed by Scotia Pharmaceuticals and others have demonstrated that EPOGAM corrects the underlying abnormality in EFA biochemistry, producing a substantial clinical improvement for many atopic eczema patients.⁶⁻⁹

NHS
PRESCRIBABLE

EPOGAM, when added to conventional symptomatic therapy, improves all aspects of atopic eczema and substantially reduces the long-term requirement for topical steroids and antihistamines.⁶⁻⁹ EPOGAM is particularly effective in relieving itch.

EPOGAM has a slow onset of action. It will be at least four to six weeks before a substantial clinical response is observed in many patients.⁶ It is not unusual for some patients to take up to 12 weeks to respond.

EPOGAM is the first oral therapy to improve atopic eczema by acting at a fundamental metabolic level.

ABRIDGED
PRESCRIBING
INFORMATION

Presentation: Oval soft capsules containing 40mg loric acid BAN in evening pill only. **Uses:** Symptomatic treatment of atopic eczema. **Dosage:** **Administration:** Adults capsules twice daily. **Children:** 1-12 years: 2-4 capsules daily. **Contra-indications:** None known. **Warnings:** **Precautions:** Use with schizophrenia, patients with epilepsy and those known epileptogenic. **Adverse Effects:** No adverse effects have been reported; nausea, indigestion, headaches have occurred.

NEW
Epogam
CAPSULES
GAMOLENIC ACID IN EVENING PRIMROSE OIL

Basic NHS
340 capsules
Product Licence H
and Number: Scotia Pl
ceuticals PL 4382/0005

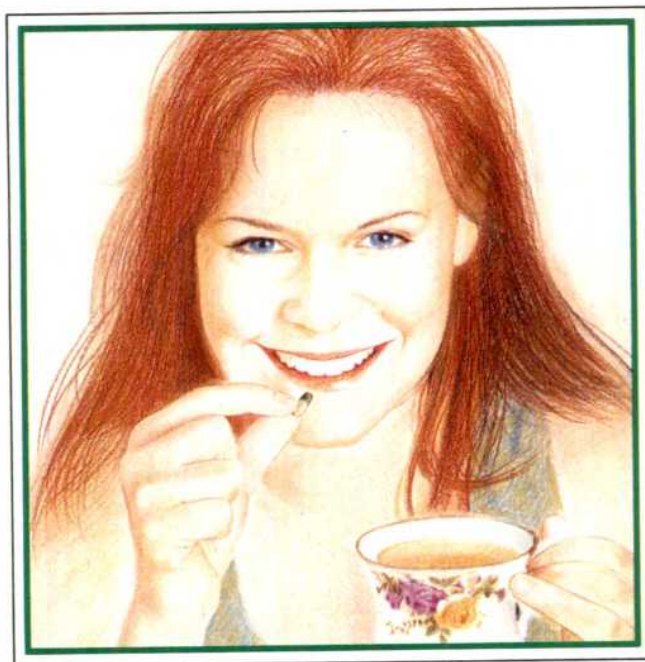
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2. Rivers JP Br Med Bull 198 59-64.
3. Manku MS et al Br J De 1984; 110: 643-648.
4. Zitoh VA & Chapkin RS Lipid Res 1988; 27: 81-105.
5. Horrobin DF J Am Dermatol, in press.
6. Stewart JCM et al Submitt
7. Wright S & Burton JL Lancet 1982; 2: 1120-1122
8. Schalin-Karrila et al Br. matol 1987; 117: 11-19.
9. Morse PF et al In Press.

Epotam, Scotia and Efarm
are registered trademarks.

IN ACNE

"Lack of patient compliance is the main cause of therapeutic failure"¹



Vibramycin^{*} 50
doxycycline ^{*}Trade Mark

A clear answer to patient compliance

Reference: 1. Cunliffe, W. J., (1981), Update Postgraduate Centre Series (Acne).



PRESCRIBING INFORMATION. Indications: Acne vulgaris. Dosage: One 50mg capsule daily, with food or milk, is recommended. Duration of treatment may vary from 6 to 12 weeks or longer dependent upon response. Contra-indications: Hypersensitivity to tetracyclines. Side Effects and Precautions: Nausea and vomiting can occur. Administration with food or milk may reduce these effects. Warnings: Administration of Vibramycin in pregnancy or to children under 8 years of age is not recommended. Staining of teeth is a possible sequel to treatment during tooth development (latter half of pregnancy or in children up to 8 years of age). Packaging: Vibramycin 50 is available as green and cream capsules, each containing 50mg of doxycycline (as the hydrochloride) in a 28 day calendar pack. Basic NHS Cost: Capsules 50mg (PL57/0238), 28 day calendar pack, £7.74.

Further information is available on request to the company:

JOURNAL OF

Official publication of

Journal of Cutaneous Pathology publishes manuscripts on dermatopathology and aims at promoting and maintaining communication. Original scientific articles on diagnostic and experimental cutaneous pathology are invited.

Clinicopathological studies will be given the highest priority.

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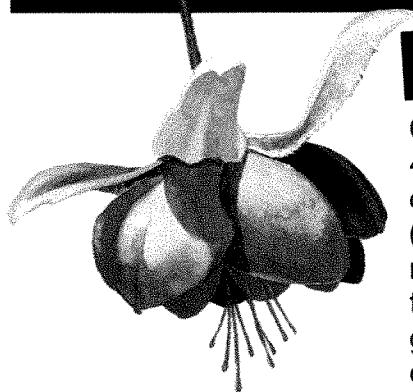
Cuplex*

Salicylic acid, Lactic acid and Copper acetate

**IN 1987 MORE DOCTORS PRESCRIBED CUPLEX
THAN ANY OTHER WART TREATMENT**

**IN 1988 MORE DOCTORS PRESCRIBED CUPLEX
THAN ANY OTHER WART TREATMENT**

whatever anyone else may tell you!



Cuplex contains only 11% salicylic acid and 4% lactic acid, yet is as effective as products containing 16.7% of both salicylic and lactic acid (1). Moreover, the self-occluding gel formulation means that messy applicators and expensive, time-consuming dressings are unnecessary - a gentle squeeze of the tube delivers Cuplex directly onto the wart and there's no risk of broken glass (always a worry with children).

Credit-card sized, plastic Compliance Cards containing clear, concise instructions for use are available on request. They also emphasise the need for regular applications, probable duration of treatment and where Cuplex can and cannot be used. They offer a durable backup to your advice and encourage correct, regular application. Thus, the treatment you prescribe is more likely to be effective.

All in all, it's hardly surprising that Cuplex is increasingly prescribed by doctors and recommended by pharmacists - it saves time and money for both patient and Health Service.

IT MAKES SENSE TO THINK OF CUPLEX FIRST

1. Bunney, M.H. et al (1976) - Br. J. Derm. **94** 667-697

CUPLEX gel PRESCRIBING INFORMATION

Presentation A clear, brownish-yellow viscous gel containing Salicylic acid Ph. Eur. 11%, Lactic acid Ph. Eur. 4%, Copper acetate (equivalent to 1.1mg of copper per 100g), Collodion, Colophony and Venice turpentine.

Uses Topical treatment of common, juvenile, plantar and mosaic warts; corns and calluses.

Contra-indications, Warnings, etc Do not apply to facial or anogenital warts. Not for use in patients sensitive to colophony. Avoid contact with the eyes. Only apply to the affected area. Keep away from naked flames.

Pharmaceutical Precautions Store in a cool place. Highly inflammable.

Legal Category P

Package Quantities 5g tubes

Further Information Most warts are contagious and any person suffering from warts should always use their own towel. Most warts will disappear after 6 to 12 weeks of treatment with Cuplex gel, providing instructions are carefully and consistently followed. Where, however, the wart continues to increase in size after 6 weeks treatment, and the patient has not consulted a doctor, the patient should be advised so to do.

Product Licence Number PL0033/0100

Basic NHS price £2.11

SMITH & NEPHEW PHARMACEUTICALS LTD., BAMPTON ROAD, HAROLD HILL, ROMFORD, ESSEX RM3 8SL,
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*Trade Mark

Smith+Nephew



A UNIQUE COMBINATION OF NATURAL MOISTURISING FACTORS

PRESCRIBING INFORMATION

Presentation

Cream containing 10% urea, 5% lactic acid, 1% hydrocortisone in an emulsified base.

Uses

Atopic eczema, allergic eczema and other inflammatory skin conditions accompanied by hyperkeratosis.

Dosage and administration

Apply twice daily after washing and drying.

Contra-indications, warnings etc.

Adverse Effects. Calmurid HC can cause smarting temporarily when applied to raw or fissured areas. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for long periods. In infants, long term continuous topical therapy should be avoided. Adrenal suppression can occur even without occlusion.

Contra-indications. Skin tuberculosis, viral infections accompanied by dermal manifestations, eg: herpes simplex, vaccinia, chicken pox, measles. Syphilitic skin lesions.

Pharmaceutical precautions

Store in a cool place but do not freeze. Do not put in alloy containers.

Legal category POM

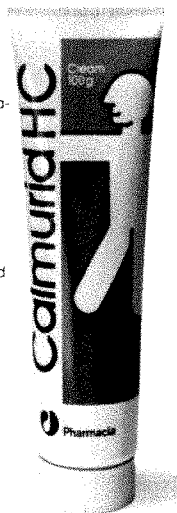
Package quantities and prices (April, 1987)

Tubes of 30 g (£2.33). Tubes of 100 g (£6.75).

Further information

Calmurid HC is a pleasant cream which does not contain lanolin or preservatives. This results in a negligible risk of allergic reactions and good patient compliance.

Product licence number 0009/5003R



Calmurid HC

Urea 10%, lactic acid 5%, hydrocortisone 1%

For dry scaly eczema



Pharmacia Pharmacia Ltd., Pharmacia House,
Midsummer Boulevard, Milton Keynes, MK9 3HP. Tel: (0908) 661101.
Further information is available on request to the Company.
Calmurid is a registered trade mark.

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- The most successful cancer book of recent years for the general physician and surgeon
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"This book is exactly what was needed. It is aimed at a wide audience — not only clinical oncologists, but also physicians, surgeons, gynaecologists, general practitioners, and even medical students. This may sound a tall order, but I believe that the authors have achieved their goal. The key to the book's success lies in a wealth of concise clinical detail written with clarity, balance and common sense ... All in all this book is destined to become a classic."

The Lancet

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Cloth **£57.50** 0 632 01373 7

BSP

Blackwell Scientific Publications
Osney Mead, Oxford OX2 0EL

The Dermatology of Bacterial Infections

G.H. Findlay

Summer 1987. 384 pages,
49 illustrations. About £50.00

The practical value of this eminently readable text is increased by the display in boxes of glossaries, epidemiology, essentials of pathology, clinical features and therapeutics. Comprehensive in its contents, it covers the field from anthrax to acne, nocardia to napkin dermatitis, rhinoscleroma to Rocky Mountain spotted fever, and tularaemia to toxic shock syndrome.

Fundamentals of Cutaneous Surgery

R.G. Bennett

Summer 1987. 864 pages,
465 illustrations.
About £59.00 Mosby

A highly illustrated and practical book providing dermatologists with a comprehensive guide to the procedures involved in cutaneous surgery.

Topical Steroids for Skin Disorders

M. Clement and A. du Vivier

1986. 80 pages,
54 illustrations. £9.95

'All in all this is a most worthwhile booklet for all those who see patients with skin disease, but who are not expert dermatologists.' *Doctor*

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**NOW IN NEW
400g PUMP DISPENSER**

RESTORES ELASTICITY TO DRY SCALY SKIN

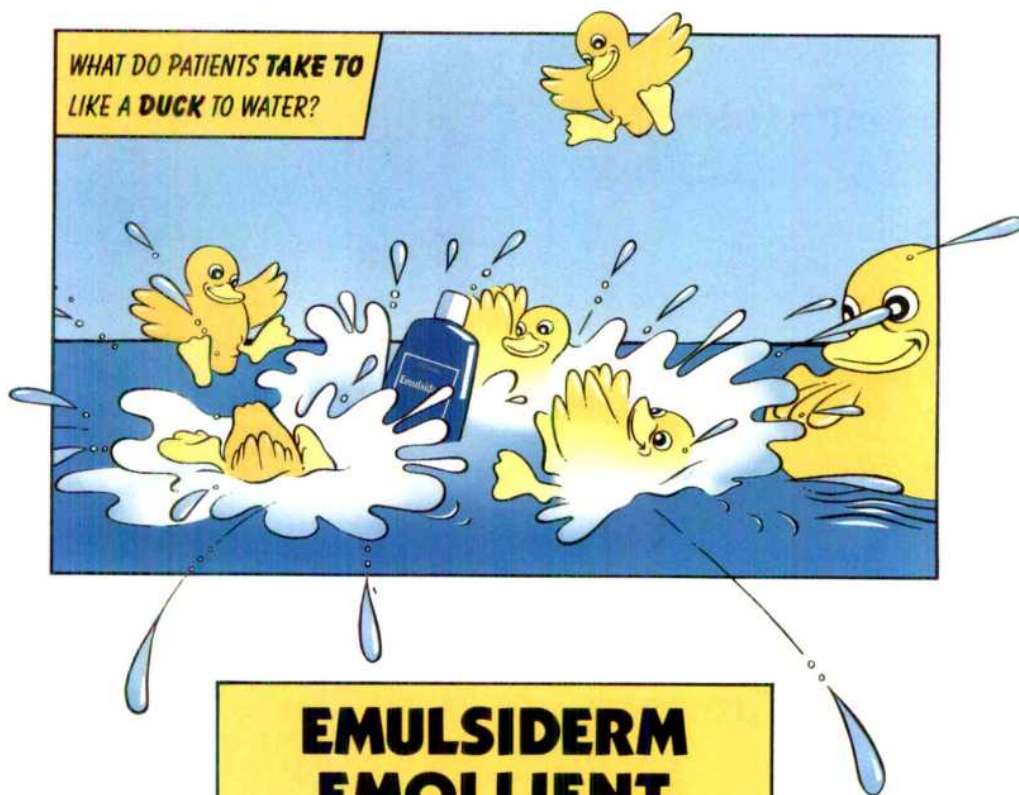
PRESCRIBING INFORMATION
Presentation Cream containing 10% urea, 5% lactic acid, in an emulsified base. **Uses** Hyperkeratosis, ichthyosis and other dry, rough scaly skin conditions. **Dosage and administration** Apply a thick layer twice daily after washing and drying. **Contra-indications, warnings etc.** Adverse Effects. Calmurid can cause smarting temporarily when applied to raw or fissured areas. **Pharmaceutical precautions** Store in a cool place but do not freeze. Do not put in alloy containers. **Legal category** G.S.L. **Package quantities and prices (March 1988)** Tubes of 50g (£1.76). Tubes of 100g (£3.49). Pump Dispenser 400g (£12.50). **Further information** Calmurid is a pleasant cream which does not contain lanolin or preservatives. This results in a negligible risk of allergic reactions and good patient compliance. **Product licence number** 0009/5009

Calmurid

Urea 10%, lactic acid 5%.

A unique combination of natural
moisturing factors.

 **Pharmacia** Pharmacia Ltd., Pharmacia House,
Midsummer Boulevard, Milton Keynes, MK9 3HP. Tel. (0908) 661101.
Further information is available on request to the Company.
Calmurid is a registered trade mark



EMULSIDERM EMOLlient

BENZALKONIUM CHLORIDE, LIQUID PARAFFIN, ISOPROPYL MYRISTATE

THE BATHTIME FAVOURITE FOR DRY SKIN PATIENTS

Emulsiderm is an effective aid in the treatment of dry skin conditions such as those associated with eczema and dermatitis. It can assist rehydrate dry skin by helping to replace lost lipids.

Being a true oil-in-water emulsion, Emulsiderm mixes easily in water, so the patient's whole body is evenly coated with emollient. And using Emulsiderm helps overcome the problem of a stubborn, messy tide-mark. No wonder so many patients prefer Emulsiderm.

Presentation Pale blue/green liquid emulsion containing 0.5% w/w Benzalkonium Chloride BP, 25.0% w/w Liquid Paraffin BP, 25.0% w/w Isopropyl Myristate BPC. **Uses** An aid in the treatment of dry skin conditions, especially those associated with eczema, ichthyosis or xeroderma. **Dosage and administration** Shake bottle before use. In the bath: Add 30 ml to a 6-8 inch bath of warm water. For infant bathing use 15 ml. Soak for 5-10 minutes. Pat dry. On the skin: Rub a small amount of undiluted emollient into the dry areas of skin until absorbed. **Contra-indications, warnings, etc** Keep away from the eyes. Take care to avoid slipping in the bath. Sensitivity to any of the ingredients. **Package quantities and basic NHS prices** Emulsiderm is supplied in polythene bottles containing 250 ml, with a 10 ml measuring cap and 1 litre, with a 30 ml measure. 250 ml £3.97, 1 litre £11.26. PL 0173/0036 PA 278/8/1

Further information is available on request Dermal Laboratories Limited, Gosmore, Hitchin, Hertfordshire SG4 7QR

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Immediate decrease in antigen-presenting function and delayed enhancement of interleukin-1 production in human epidermal cells after *in vivo* UVB irradiation

L.RÄSÄNEN, T.REUNALA*, M.LEHTO, C.JANSÉN†, I.RANTALA‡ AND P.LEINIKKI

Departments of Biomedical and *Clinical Sciences, University of Tampere, ‡Department of Pathology, University Central Hospital of Tampere and †Department of Dermatology, University of Turku, Finland

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SUMMARY

Human skin was irradiated *in vivo* with a single UVB dose (100 mJ/cm² or 200 mJ/cm²) to examine simultaneously the antigen-presenting function and interleukin-1 (IL-1) production capacity of irradiated epidermal cells (EC). Suction blisters were produced on irradiated areas on days 0, 3 and 7 after UVB. Irradiated EC were harvested and co-cultured with autologous T lymphocytes in the presence of antigens (PPD, HSV) or mitogen (ConA). Culture supernatants were tested for IL-1 activity using the thymocyte comitogenity assay. We found that a single 200 mJ/cm² dose of UVB caused an immediate suppression of the antigen-presenting function of EC, but no alteration in their IL-1 production capacity or surface marker expression (ATPase, CD1). PPD- and HSV-induced lymphocyte proliferation was decreased 70–80% and ConA-driven proliferation 30% when compared to non-irradiated EC. However, this suppression was restored on days 3 and 7 after UVB irradiation, this being coexistent with an increased capacity of EC to produce IL-1. It remains to be elucidated whether the immediate UVB-induced photoimmunosuppression observed in the present study is due to inhibitory mediators or impaired membrane function of EC or both.

Langerhans cells (LC) are epidermal antigen-presenting cells capable of inducing allogenic, antigen-specific, and cytotoxic T cell proliferation.^{1,2} This functional capacity of LC can be altered by various physicochemical agents, including ultraviolet (UV) light. In mice, UVB irradiation of epidermal cells (EC) *in vitro* causes impaired antigen presentation and irradiation of murine skin induces systemic suppression of contact hypersensitivity which is unrelated to

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the diminished LC surface marker expression.^{3,4} In man, there is evidence that UVB given both *in vitro* and *in vivo* causes a dose-dependent decrease in the antigen-presenting and alloactivating capacity of EC or enriched LC fractions.^{5,6} In addition to LC, keratinocytes represent another target for UV irradiation. Both of these cells can release a cytokine, interleukin-1 (IL-1), which may participate in the regulation of UV-mediated local, as well as systemic immunosuppression and inflammatory reactions.⁷⁻¹⁰

The present study was performed to investigate whether a single low-dose UVB irradiation of human skin depresses EC antigen-presenting function and has simultaneous effects on IL-1 production. The timing is important and, therefore, we examined these functions several days after UVB exposure and correlated the findings also to LC surface marker expression.

METHODS

Patients, irradiations and suction blisters

Eleven dermatological patients (seven with discoid eczema, two with psoriasis and two with nodular prurigo) volunteered for the study. Their mean age was 38 years (range 28–52 years). Ten had skin type III, one had skin type II and none of them suffered from light sensitivity. No systemic treatment was given and the abdominal skin was free of skin lesions and had no sun-exposure for at least 2 weeks prior to UVB irradiation.

UVB was given on one or two 150 cm² test areas of uninvolved abdominal skin using a Waldmann UV 8001 K cabin (Waldmann GmGH & Co., Schwenningen, F.R.G.) with 18 UVB (Voltarc-2072) bulbs emitting in the 285–355 nm range with a maximum at 315 nm. The irradiance of each bulb was 2.2 mW/cm² and the test areas were given either a single 100 mJ/cm² or 200 mJ/cm² dose of UVB.

In five patients suction blisters were raised immediately after irradiation (day 0) on the irradiated and contralateral non-irradiated abdominal skin.¹¹ Fully developed blister roofs were obtained 100–150 min after UVB irradiation. In six patients suction blisters were performed on day 0 and either on day 3 or day 7 after a single 200 mJ/cm² dose of UVB in order to examine the delayed irradiation effects. Due to the many samples taken from irradiated skin no control samples from non-irradiated skin could be obtained from these six patients. Therefore, control suction blisters were raised on the uninvolved abdominal skin of 18 dermatological patients aged 27–56 years who were not receiving any UVB.

Isolation and cultures of epidermal cells and T lymphocytes

Epidermal sheets were taken from suction blister roofs and these were enzyme-treated as previously described.¹² In brief, the sheets were treated with 0.25% trypsin (Gibco Ltd., Paisley, Scotland U.K.) for 30 min at 37°C and then with 0.01% DNase I (Sigma Chemical Co., St. Louis, MO, U.S.A.) for 5 min at room temperature, after which the cell suspension was filtered through a nylon mesh.

Venous citrated blood was centrifuged by Ficoll-Isopaque and T lymphocytes were purified from the mononuclear cells by rosette formation with aminoethylisothiuronium bromide-treated sheep erythrocytes as previously described.¹³ The T-cell populations contained <0.1% monocytes as assessed by non-specific esterase staining, <3% OKB7-positive cells (B lymphocytes) and >90% OKT3-positive cells (T lymphocytes).

UVB-irradiated and non-irradiated EC and autologous T lymphocytes were suspended at a density of 0.5×10^6 cells/ml in RPMI 1640 supplemented with 20% autologous plasma. Thereafter, 50 µl T cells, 50 µl EC and 100 µl stimulants in RPMI 1640 were added per well of

V-bottomed microplates. The stimulants used were purified protein derivative of tuberculin (PPD, Statens Seruminstitut, Copenhagen, Denmark), Herpes simplex virus type 1 (HSV-1, Behringwerke AG, Marburg, F.R.G.) and concanavalin A (ConA, Pharmacia Fine Chemicals, Uppsala, Sweden). The concentrations used were 1 µg/ml (PPD), 1/1000 dilution of a stock preparation (HSV) and 12.5 µg/ml (ConA), and the cultures were terminated after 6 days. Sixteen hours before harvesting 0.125 µCi of iododeoxyuridine (Amersham, Buckinghamshire, England, U.K.) containing 1 µM fluorodeoxyuridine was added per well¹⁴ and the uptake of the isotope was measured with a gamma counter.

Measurement of interleukin-1

For the production of IL-1, UVB-irradiated and non-irradiated EC (10⁶ cells/ml) were incubated in 5% autologous plasma-RPMI 1640 in tissue culture tubes for 2 days. To enhance the elaboration of IL-1, formalin-treated *Staphylococcus epidermidis* bacteria (bacterium-to-epidermal cell ratio 2:1) were added to the cultures. IL-1 activity in the culture supernatants was determined as described by Luger *et al.*¹⁵ using the thymocyte mitogenicity assay.

Examination of Langerhans cells

Sheets from suction blister roofs were stained with both the ATPase and the immunoperoxidase method using CD1 antiserum (OKT6, Ortho Diagnostic Systems, Raritan, NJ, U.S.A.) as previously described.¹⁶ In addition to LC counting with light microscopy, routine transmission electron microscopy (EM) was performed. Blister roofs taken on days 0, 3 and 7 were fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon. Ultra-thin sections were then stained with lead citrate and uranyl acetate and viewed with a Jeol JEM-1200EX electron microscope.

RESULTS

Accessory function of epidermal cells after UVB

In preliminary experiments the immediate effects of 100 mJ/cm² and 200 mJ/cm² doses of UVB were compared. Figure 1 depicts the results obtained with two volunteers. A slight decrease in the accessory function of EC was caused by 100 mJ/cm² when compared to non-irradiated EC from the same patient. A more evident drop occurred when a dose of 200 mJ/cm² was given. The HSV- and PPD-induced lymphocyte proliferation was 73–79% and ConA-induced proliferation was 26% lower than that obtained with non-irradiated EC.

Table 1 shows the effects of a single 200 mJ/cm² dose of UVB on days 0, 3 and 7. The decreased PPD- and HSV-induced proliferative responses observed on day 0 increased with time. On days 3 and 7 the responses were about 10 times higher and were comparable to those of 18 non-irradiated control patients. On days 3 and 7 Con A stimulation was about two times higher than on day 0. The mean cpm values from 18 non-irradiated control individuals were for PPD 2840 (range 940–8180), for HSV 3840 (920–6740) and for ConA 4700 (1510–10250), respectively. The c.p.m. values of non-stimulated control cultures were < 100.

Interleukin-1 production after UVB

IL-1 activity in the culture supernatants from irradiated and non-irradiated EC taken immediately after UVB (day 0) was similar and no marked difference was found between 100 mJ/cm² and 200 mJ/cm². In five patients the mean IL-1 activity of irradiated EC was 113% (range 83–170%) of that of non-irradiated EC. The mean IL-1 activity of non-irradiated EC in

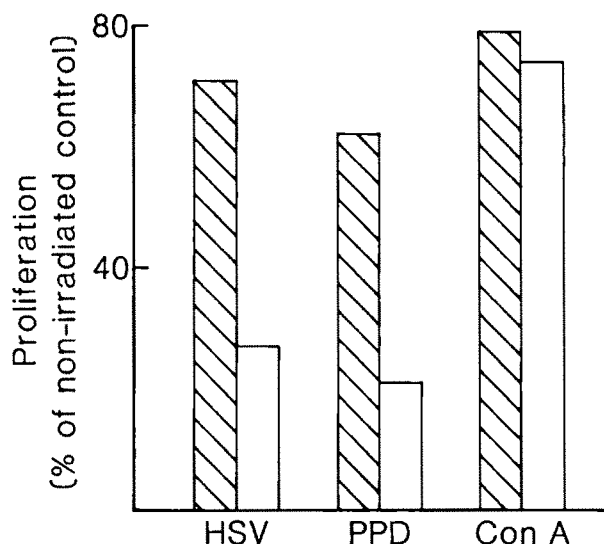


FIGURE 1. Immediate effect of UVB on accessory function of epidermal cells. Normal abdominal skin of two volunteers was irradiated with 100 mJ/cm² (■) or 200 mJ/cm² (□) and freshly prepared epidermal cells were cocultured with autologous T cells by using three different stimulants. T-cell proliferation was measured on day 6 and the results were compared to those of non-irradiated epidermal cells from the same individual.

these patients was 14.1 U/ml. Table 2 shows the IL-1 production capacity of EC when the cultures were set up on days 0, 3 and 7 after 200 mJ/cm² dose of UVB. When compared to day 0 a clear enhancement was observed on day 3 and day 7. Interestingly, the highest IL-1 activities were found in those two patients, one with skin type II and second with skin type III, who had profound erythema and oedema on UVB-irradiated skin areas.

TABLE 1. Effect of a single *in vivo* 200 mJ/cm² UVB dose on accessory function of epidermal cells in six human volunteers. Irradiated epidermal cells harvested from suction blister roofs were cocultured with T cells in the presence of three stimulants for 6 days

Immediately after irradiation			3 days after irradiation			7 days after irradiation		
PPD	HSV	ConA	PPD	HSV	ConA	PPD	HSV	ConA
160*	-†	5230	1230	-	210	n.d.	n.d.	n.d.
100	60	2490	1730	900	230	n.d.	n.d.	n.d.
460	230	3250	4670	4590	930	n.d.	n.d.	n.d.
130	510	3510	n.d.	n.d.	n.d.	2200	2560	5740
540	160	3220	n.d.	n.d.	n.d.	9480	2790	5990
360	-†	5420	n.d.	n.d.	n.d.	2460	-	7820

* = incorporation of iododeoxyuridine (c.p.m.).

† = seronegative for HSV.

n.d. = not done.

TABLE 2. Effect of a single *in vivo* 200 mJ/cm² UVB dose on generation of interleukin-1 in epidermal cell cultures from six human volunteers

Immediately after irradiation	3 days after irradiation	7 days after irradiation
15*	36	n.d
19	44	n.d
8	73	n.d
12	n.d	27
25	n.d	46
10	n.d	49

* = units/ml.

n.d = not done.

Effects of UVB on Langerhans cells

No immediate effect of UVB was observed on LC markers. The cell densities were similar on day 0 both after 100 mJ/cm² and 200 mJ/cm² of UVB. The mean densities were 609 and 669 cells/cm² as assessed by the ATPase and CD1 immunoperoxidase methods, respectively. These figures were 89–99% of those found in non-irradiated skin from the same five patients. Blister roofs taken on days 3 and 7 after UVB showed a marked reduction in ATPase and CD1-positive cell numbers (Table 3). The cells showed shortened dendritic processes and more rounded general appearance and on day 3 the figures were 55% and 57%, and on day 7, 38% and 39% of those counted on day 0, respectively.

EM examination showed no damage to LC in the irradiated and non-irradiated blister roofs taken on day 0. No evidence of LC damage was found in day 3 and day 7 samples and it appeared that no depletion of LC occurred.

TABLE 3. Effect of a single *in vivo* 200 mJ/cm² UVB dose on surface markers of Langerhans cells in six human volunteers

Immediately after irradiation		3 days after irradiation		7 days after irradiation	
CD1-immunoperoxidase	ATPase	CD1-immunoperoxidase	ATPase	CD1-immunoperoxidase	ATPase
717*	628	493	455	n.d	n.d
712	768	435	401	n.d	n.d
485	449	202	182	n.d	n.d
n.d	520	n.d	n.d	n.d	208
805	742	n.d	n.d	392	309
627	545	n.d	n.d	179	178

* = cells/mm² in suction blister roofs.

n.d = not done.

DISCUSSION

In the present study, healthy abdominal human skin was irradiated with a low-dose of UVB *in vivo*, after which accessory cell function, IL-1 production and membrane marker expression of EC were determined. We observed that the cells harvested within 2 h after a single 200 mJ/cm² dose of UVB were clearly less efficient in antigen presentation than non-irradiated EC, while no change was found in their IL-1 production capacity or ATP-ase and CD1 expression. These results resemble those of Cooper *et al.*⁶ who irradiated human volunteers with 100 to 200 mJ/cm², i.e. with two to four minimal erythema doses of UVB. They documented immediate inhibition of EC alloantigen presentation and, in agreement with the present results, observed no concomitant decrease in LC membrane marker expression. It is thus not clear what causes the rapid suppression of the EC accessory function after *in vivo* UVB irradiation. It is possible that UVB directly causes some alteration in the membrane function of LC. Interestingly, recent *in vitro* studies have provided evidence that the expression of membrane-bound IL-1 is inhibited by UVB on rat alveolar macrophages¹⁷ but at present it is obscure whether UVB irradiation impairs IL-1 expression on human LC. UVB-induced inhibition of LC antigen-presenting function occurs very rapidly and it could also be mediated by soluble suppressive factors such as prostaglandins,¹⁸ *cis*-urocanic acid,¹⁹ IL-1 inhibitors²⁰ and oxygen intermediates,²¹ all factors that have been connected with photoimmunosuppression. However, Cooper *et al.*⁶ presented evidence that the immediate UVB-induced inhibition in LC alloantigen presentation was not reversible with exogenous indomethacin or human IL-1.

The timing is important when investigating UVB-induced effects on human skin. We harvested EC immediately or on days 3 and 7 after irradiation. An interesting finding was that after 3 and 7 days, from a single 200 mJ/cm² dose of UVB, the initially low responses to various stimulants reverted to a normal level, although the population densities of ATPase- and CD1-positive cells were now decreased. This morphological and functional discrepancy after *in vivo* UV irradiation has also been previously documented in murine and human studies.^{6,22} Refined EM examinations have revealed loss of membrane markers but no ultrastructural damage in murine LC even after high-dose UVB irradiation.²³ In agreement with this, we did not observe any evident EM alterations in LC examined either on day 3 or 7 when the number of ATPase and CD1 positive cells were decreased. However, the normal or even enhanced function of EC several days after an *in vivo* UVB exposure does not exclude decreased viability or impaired function of the cells originally irradiated with UVB. New cells may migrate to the epidermis and participate in immune functions and interestingly, increased amounts of CD1⁻ DR⁺ cells distinct from LC have been observed in human epidermis after UVB exposure.^{24,25}

There is evidence that both keratinocytes and LC form soluble IL-1. LC produce higher activities than keratinocytes on a cell basis, but since LC are normally few in number in the epidermis the greater part of epidermal IL-1 activity seems to be derived from keratinocytes.^{7,12} Interestingly, UVB causes augmented IL-1 expression in cultured human keratinocytes and in UVB-irradiated human epidermis, suggesting that IL-1 may mediate the local inflammation and other acute phase responses characteristic of UVB-injured skin.^{9,10} In agreement with this, we found increased IL-1 activities in irradiated EC culture supernatants on days 3 and 7 when compared with the activities immediately after UVB. The individual responses were not very marked but the increase was evident in every one of the six patients examined and the highest IL-1 activities were observed in those two patients who exhibited the most intense erythema reactions.

In conclusion, the present findings confirm that low-dose UVB irradiation given *in vivo* has a

prominent immediate effect on antigen-presenting function and a delayed effect on IL-1 production of human EC when assessed by *in vitro* techniques. The observed functional photoimmunosuppression was restored a few days after irradiation and this occurred together with augmented epidermal cell IL-1 production capacity.

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DNA analysis by flow cytometry in cutaneous T-cell lymphomas

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SUMMARY

DNA histograms of skin and blood specimens from 64 patients with known or suspected cutaneous T-cell lymphoma (CTCL) have been examined and compared with normal blood mononuclear cells and skin biopsy samples from 50 patients with various benign cutaneous conditions (i.e. patch test infiltrates, eczema, psoriasis, lichen planus, atopic dermatitis) in an attempt to establish whether DNA measurements by flow cytometry may improve the early recognition of CTCL. The results indicate that right-skewed G₀/G₁ peaks are seen frequently in both benign disorders and known and suspected CTCL. Such peaks may reflect increased stainability of DNA due to chromatin dispersion during cell activation and/or cell proliferation and do not constitute reliable evidence of malignancy. In contrast, discrete aneuploid DNA peaks are confined to malignant lesions, but are seen almost exclusively in the advanced stages in which the diagnosis can be established easily based on routine histological criteria. These data indicate that DNA measurements by flow cytometry is of only limited help in the early recognition of CTCL and support the view that the lymphoid infiltrate in early CTCL may be reactive (rather than neoplastic) or alternatively may contain only minor populations of abnormal (malignant) cells which cannot be detected by currently available DNA measurement techniques.

The cutaneous T-cell lymphomas (CTCL) are a heterogeneous spectrum of disorders of which mycosis fungoides (MF) and Sézary syndrome (SS) are the best defined clinical entities.¹ These conditions may be confined to the skin for long periods and are often preceded by indolent or so-called pre-malignant phases which are difficult or impossible to distinguish reliably from benign cutaneous lymphoid infiltrates by routine histological criteria.² However, they progress

almost invariably to more severe, disseminated lesions which usually do not present any major diagnostic difficulty. However, at that stage they are unfortunately often resistant to therapy.³ For these reasons, the early recognition of CTCL has attracted considerable attention, and much effort has been directed towards the search for alternative diagnostic criteria, e.g. ultrastructural and immunophenotypic.⁴⁻⁶ However, none of these has proved reliable in routine practice.

DNA measurements by cytophotometry or flow cytometry have provided a useful supplement to the diagnosis of leukaemias and nodal lymphoid malignancies.⁷ It has been proposed that these methods may also be helpful in the early recognition of CTCL.^{8,9} Results so far have, however, not been consistent, and at present the value of DNA measurements in the early recognition of CTCL is not clear.¹⁰⁻¹²

In this study, skin and blood samples from 64 patients with known or suspected CTCL have been examined and compared with data published previously on benign cutaneous lymphoid infiltrates^{10,11} in an attempt to establish whether DNA measurements by flow cytometry may improve the early diagnosis of CTCL.

METHODS

Biopsy samples

Skin biopsy samples and blood specimens were obtained from 64 patients with known or suspected CTCL. Forty-one patients were followed by serial studies during follow-up periods of between 3 and 36 months, and a total of 213 skin biopsy samples and 76 blood specimens were available for analysis.

In all cases, diagnosis was based on standard clinical and histological criteria.¹³ All available histopathological specimens were re-evaluated by one of the authors (E.R.). In MF, the staging system proposed by the Scandinavian Mycosis Fungoides Co-operative Group was adopted: stage I = clinically suspicious, histologically non-diagnostic poikilodermic plaques or erythrodermic lesions; stage II = plaque lesions of MF, no extracutaneous disease; stage III = tumour lesions of MF, no extracutaneous disease; and stage IV = MF with involvement of lymph nodes, showing on histology, either dermatopathic lymphadenopathy (IVa) or malignant lymphoma (IVb).³

The controls were blood mononuclear cells from 16 adult healthy volunteers (eight males and eight females) aged 26-53 years (mean 36 years), and skin biopsy samples from 50 patients with various benign cutaneous conditions, i.e. patch test infiltrates (18 cases), psoriasis (14 cases), lichen planus (seven cases), atopic dermatitis (five cases), and chronic eczema (six cases).

DNA flow cytometry

Skin biopsies and Lymphoprep® isolated blood mononuclear cells were frozen and stored in liquid nitrogen. Before DNA analysis, the epidermis was separated by a tangential cut through the frozen biopsy. The dermal specimens were cut into pieces smaller than 1 mm, placed in phosphate buffered saline with 1% (v/v) fetal calf serum, shaken vigorously for 20 s, filtered through a 100 µm filter and then centrifuged.⁹ The sediment was resuspended in hypotonic buffer solution, containing detergent (Nonidet P40, 1% (v/v)), propidium iodide (50 mg/l), and ribonuclease (Sigma type 1A, 100 mg/l). The nuclear suspension was allowed to stain for 15 min, and was then filtered through a 30 µm filter. Finally, a similar stained preparation of trout and chicken erythrocyte nuclei was added to provide internal DNA standards.¹⁴

In each sample, the DNA content of at least 50,000 nuclei was measured with and without

internal standards in a Becton Dickinson FACS IV flow cytometer. The DNA distributions were deconvoluted by maximum likelihood, assuming uniform S-phase distributions.¹⁵ Right-skewed peaks were fitted with an extra Caussian to the right of the main Go/G1 peak.

The DNA indices were calculated as follows. The patient sample peak was divided by the simultaneously measured trout peak, and this value was divided by the mean value of normal male or female lymphocytes relative to trout erythrocytes. All values were corrected for nonlinearity.

RESULTS

A distinction could be made between three main types of DNA histograms depending upon the DNA index of the main population of Go/G1 cells and upon the presence or absence of discrete aneuploid DNA peaks, i.e.:

- (i) normal DNA histograms in which the DNA index of the main population of diploid (Go/G1) cells was within the 1% confidence limits (1 ± 0.036) for normal blood mononuclear cells. In these specimens discrete aneuploid peaks were not identified.
- (ii) DNA histograms with right-skewed Go/G1 peaks with DNA indices above the 1% upper confidence limit (1.036) for normal blood leucocytes. In some of these cases, an additional peak was fitted with an extra Caussian to the right of the main peak. However, discrete aneuploid peaks were not identified.
- (iii) DNA histograms with discrete aneuploid peaks which in contrast to the estimated additional peaks were clearly separated from the normal Go/G1 cells.

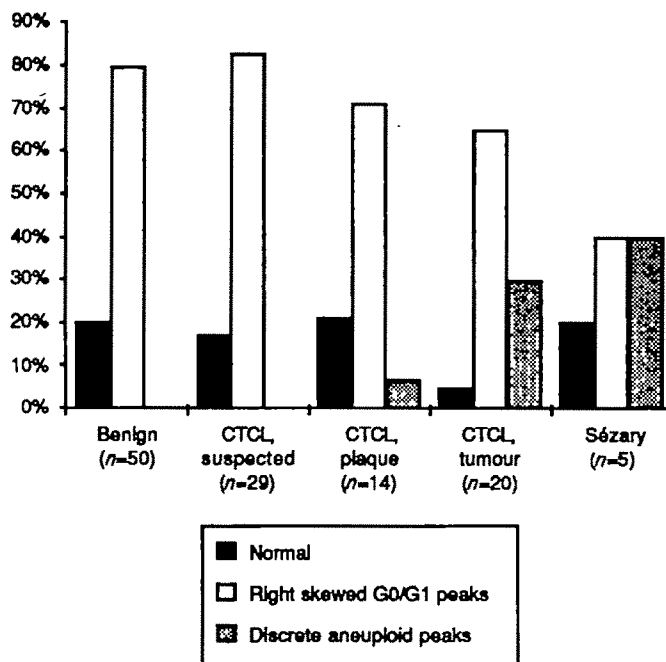


FIGURE 1. DNA histograms in known and suspected CTCL compared with benign cutaneous conditions.

TABLE 1. DNA indices (mean (and range)) in skin biopsy samples from known and suspected CTCL compared with benign cutaneous conditions

Diagnosis	Right-skewed Go/G1 peaks				
	Normal Go/G1 peaks*	One peak	Two peaks†		Discrete aneuploid peaks
			Main peak	Additional peak	
Benign (n = 50)	1.032 (1.026-1.036)	1.047 (1.037-1.066)	1.052 (1.027-1.089)	1.14 (1.10-1.19)	
MF, suspected (n = 29)	1.022 (0.967-1.033)	1.045 (1.037-1.047)	1.035 (1.002-1.060)	1.12 (1.08-1.19)	
MF, plaque (n = 14)	1.016 (0.981-1.034)	1.045 (1.041-1.150)	1.036 (1.021-1.066)	1.11 (1.10-1.14)	2.03
MF, tumour (n = 20)	1.012 (0.987-1.033)	1.072 (1.049-1.128)	1.052 (0.987-1.073)	1.16 (1.08-1.18)	1.53 (1.15-1.77)
Sézary syndrome (n = 5)	1.011 (0.953-1.036)	1.042 (1.038-1.053)	1.040 (1.019-1.066)	1.15 (1.11-1.19)	1.98 (1.94-2.00)

* The coefficient of variation (indicating the resolution of the staining method and the flow cytometer) ranged from 1.70 to 4.45% (mean 2.68%).

† In these cases, an additional peak was fitted with an extra Gaussian to the right of the main peak in order to compensate for skewness.

The distribution of patients in these categories is shown in Figure 1. The DNA indices are listed in Table 1, and representative examples are illustrated in Figure 2.

Benign cutaneous conditions

The results from the study of skin biopsy samples from benign cutaneous conditions have been described in detail elsewhere.^{10,11} In brief, normal DNA histograms were seen in 10 cases. The cutaneous specimens from the remaining 40 benign disorders showed right-skewed Go/G1 peaks. One right-skewed peak with DNA indices of between 1.037 and 1.066 (mean 1.047) was seen in 17 cases. In the remaining 23 cases, an additional peak, accounting for 4-22% of the cells, could be estimated (see Table 1). The DNA indices of these peaks ranged from 1.10 to 1.19 (mean 1.14). Discrete aneuploid DNA peaks were not identified in any of the specimens. The proportion of cells in the S and G2/M phases ranged from 5-25% (mean 10.3%) and 2-6.5% (mean 3.6%), respectively (see Table 2).

MF, suspected (stage I)

A total of 56 skin biopsy samples from 29 patients were studied. Normal DNA histograms were seen in five patients (see Fig. 1). The remaining 24 patients showed right-skewed Go/G1 peaks similar to those described above. One right-skewed peak with DNA indices of between 1.037 and 1.047 (mean 1.045) was seen in five cases. In the remaining 19 cases, an additional peak, accounting for 7 to 17% of the cells, could be estimated (see Table 1). The DNA indices of these peaks ranged from 1.08 to 1.19 (mean 1.12).

Measurement of blood samples from five patients showed normal DNA histograms in four patients. In the remaining patient, the DNA index of the main population of diploid cells was increased (1.044) relative to that of normal blood mononuclear cells. Discrete aneuploid DNA peaks were not identified in any of the specimens.

In the skin biopsy samples, the proportion of cells in the S and G2/M phases ranged from 1.3-17.1% (mean 12.3%) and 1.9-9.5% (mean 3.6%), respectively. The blood mononuclear cells showed a lower proliferative rate with a mean of 5.6% and 1.9% of the cells in the S and G2/M phases, respectively (see Table 2).

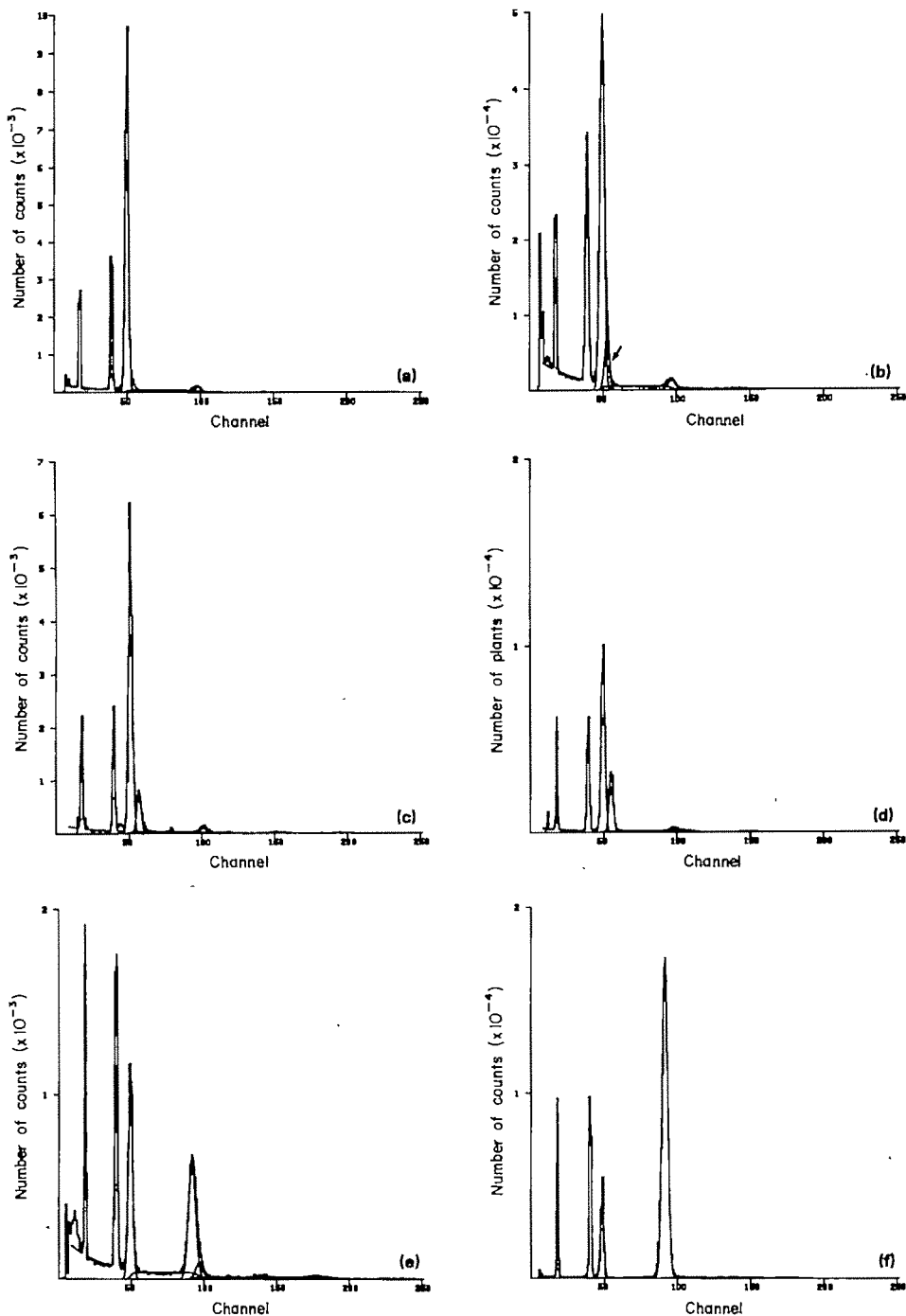


FIGURE 2. DNA histograms from suspected CTCL (a) and (b), tumour lesions of CTCL (c) and (d) and skin (e) and blood (f) specimens from Sézary syndrome. The first peaks indicated the internal reference standards, i.e. chicken and trout erythrocytes. The patients' samples showed a normal DNA histogram in (a); and a right-skewed Go/G1 peak with an estimated additional peak (arrow) in (b). Discrete aneuploid DNA peaks in the hyperdiploid or tetraploid region are seen in (c), (d), (e) and (f). In contrast to the estimated additional peak (arrow in b), such peaks were clearly separated from the normal Go/G1 cells.

TABLE 2. Cell cycle distributions (mean (and range)) in known and suspected CTCL compared with benign conditions

Diagnosis	Specimen	Go/G1 (%)	S (%)	G2/M (%)
Benign	Skin	86.2 (72.0-91.0)	10.3 (5.0-25.0)	3.6 (2.0-6.5)
MF, suspected	Skin	86.2 (79.6-90.6)	12.3 (1.3-17.1)	3.6 (1.9-9.5)
	Blood	92.4 (85.4-98.5)	5.6 (1.4-14.4)	1.9 (0.1-2.8)
MF, plaque	Skin	83.8 (76.3-88.2)	12.3 (8.4-19.7)	4.2 (3.0-7.5)
	Blood	89.0 (84.5-97.0)	7.9 (2.3-12.9)	1.8 (0.1-6.4)
MF, tumour	Skin	84.8 (79.1-98.3)	11.8 (8.0-17.9)	3.9 (2.1-5.5)
	Blood	90.6 (82.4-98.7)	6.6 (1.2-16.3)	1.2 (0.1-3.8)
Sézary syndrome	Skin	82.8 (73.2-90.4)	11.8 (6.3-16.7)	5.6 (4.0-10.0)
	Blood	93.5 (82.3-97.5)	4.8 (1.9-11.6)	1.6 (0.1-7.5)

MF, plaque (stage II)

A total of 32 skin samples from 14 patients were studied. A discrete aneuploid peak, accounting for 19% of the cells, was seen in one patient. The remaining 13 patients showed either normal DNA histograms (three cases) or right-skewed Go/G1 peaks (10 cases). One right-skewed Go/G1 peak with DNA indices of between 1.041 and 1.150 (mean 1.045) was seen in three cases. In the remaining seven cases an additional peak, accounting for 2-16% of the cells, could be estimated. The DNA indices of these peaks ranged from 1.10 to 1.14 (mean 1.11).

Measurements of blood samples from 12 patients showed normal DNA histograms in eight patients. The remaining four patients showed right-skewed Go/G1 peaks with DNA indices of between 1.037 and 1.059 (mean 1.045). Discrete aneuploid DNA peaks were not identified in any of the blood specimens.

The proportion of cells in the S and G2/M phases was similar to that seen in benign disorders and suspected CTCL (see Table 2).

MF, tumour (stages III and IV)

A total of 79 skin biopsies from 20 patients were studied. Discrete aneuploid DNA peaks, accounting for 9-20% of the cells, were seen in six patients (see Table 1 and Figs 1 and 2). The DNA indices of these peaks ranged from 1.15 to 1.77 (mean 1.53). The remaining 14 patients showed either normal DNA histograms (one patient) or DNA histograms with right-skewed Go/G1 peaks (13 patients). One right-skewed peak with DNA indices of between 1.049 and 1.128 (mean 1.072) was seen in six cases. In the remaining seven cases, an additional peak, accounting for 3-34% of the cells, could be estimated. The DNA indices of these peaks ranged from 1.08 to 1.18 (mean 1.16).

Measurements of blood samples from 12 patients showed normal DNA histograms in seven patients. The remaining five patients showed right-skewed Go/G1 peaks with DNA indices of between 1.037 and 1.058 (mean 1.047). Discrete aneuploid DNA peaks were not identified in any of the blood specimens.

The proportion of cells in the S and G2/M phases was similar to that described above (see Table 2).

Sézary syndrome

A total of 40 skin biopsies and 26 blood samples from five patients were studied. Normal DNA

histograms were seen in one patient. The remaining four patients showed either right-skewed Go/G1 peaks (two patients) or discrete aneuploid peaks (two patients) in both blood and skin biopsy samples (see Table 1 and Figs 1 and 2).

The proportion of cells in the S and G2/M phases ranged from 6.3–16.7% (mean 11.8%) and 4–10% (mean 5.6%) in the skin biopsy samples, and from 1.9–11.6% (mean 4.8%) and 0.1–7.5% (mean 1.6%) in the blood specimens (see Table 2).

DISCUSSION

Abnormalities of DNA content in CTCL were first described by Lutzner *et al.*¹⁶ who showed that two variants of Sézary syndrome may be distinguished, i.e. a large cell variant characterized by tetraploid or near-tetraploid cellular DNA, and a small cell variant with normal or near-normal DNA index values. Since then there has been much progress in the understanding of CTCL, and it is now evident that these conditions may show non-random chromosome abnormalities,^{17,18} and that abnormal cellular DNA is not confined to the Sézary syndrome, but may also be seen in MF.^{8,9,12,19} There are some indications that DNA measurements may be relevant for prognostic and/or therapeutic reasons,¹⁹ and there have been many suggestions that these methods may also help in the early diagnosis of CTCL.^{8,9} This view has, however, not been well supported by more recent studies which have suggested that DNA histogram abnormalities similar to those seen in early CTCL are not uncommon in benign cutaneous conditions.^{10,11}

In this study, skin and blood samples from 64 patients with known or suspected CTCL have been examined and compared with normal blood mononuclear cells and skin biopsy samples from benign cutaneous conditions in an attempt to establish whether DNA measurements by flow cytometry may improve the early diagnosis of CTCL.

The results indicate that two types of DNA histogram abnormalities may be distinguished: DNA histograms with discrete aneuploid peaks which are clearly separated from the normal Go/G1 cells; and DNA histograms with right-skewed Go/G1 peaks with DNA indices above the 1% upper confidence limit for normal blood mononuclear cells. The former (discrete aneuploid) peaks were confined to malignant infiltrates, but were seen relatively rarely and mainly in the advanced stages in which the diagnosis could be established easily based on routine histological criteria. In contrast, the latter (right-skewed Go/G1) peaks were seen frequently in all stages of CTCL. Unfortunately, however, such peaks did not constitute reliable evidence of malignancy, as similar peaks were also seen in a substantial number of biopsy specimens from benign cutaneous conditions.

In a previous study, it has been proposed that right-skewed Go/G1 peaks may be indicative of the presence of minor populations of abnormal (malignant) cells.⁹ This possibility cannot be formally excluded, and it is well known that minor DNA differences may be extremely difficult to detect by flow cytometry, especially in cases of mixed cell populations.²⁰ However, alternative explanations clearly must be sought to account for the frequent presence of such peaks in benign cutaneous lymphoid infiltrates.

It is possible that right-skewed Go/G1 peaks may be artefactual, i.e. may result from staining of incompletely lysed RNA and/or from clumping of nuclei during the preparation procedures.¹⁰ However, against this possibility is the fact that consistent DNA content estimations have been obtained by serial studies of individual patients,⁹ and that right-skewed Go/G1 peaks have never been identified in normal blood leucocytes, as would be expected if artefacts were implicated. An alternative possibility is that right-skewed diploid peaks may reflect altered

stainability of DNA due to chromatin dispersion during cell activation and cell proliferation.^{10,21} In keeping with this assumption are the results from previous cell kinetic measurements in CTCL²² and also the observation that benign and malignant cutaneous T-cell infiltrates frequently express activation and proliferation associated antigens, e.g. HLA-DR, interleukin-2 receptor (CD25) and the Ki-67 cell proliferation associated nuclear antigen.^{23,24}

In summary, the present study indicates that advanced CTCL may show discrete aneuploid DNA peaks and that such abnormalities are confined to malignant infiltrates. In contrast, lesions of early suspected CTCL and most cases of plaque stages cannot be distinguished from benign cutaneous lymphoid infiltrates by single cell DNA measurements by flow cytometry. These findings support the view that early CTCL may be reactive (rather than neoplastic) or alternatively may contain only minor populations of malignant cells²⁵ which cannot be detected by currently available DNA measurement techniques. This concept may help to explain the indolent course and favourable prognosis seen in many early CTCL, and is in keeping with the observation that early CTCL does not contain detectable monoclonal T-cell populations²⁶ and are indistinguishable from benign cutaneous lymphoid infiltrates in terms of their immunophenotypic properties.^{5,6}

ACKNOWLEDGMENTS

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Peripheral blood mononuclear leukocyte cyclic adenosine monophosphate specific phosphodiesterase activity in childhood atopic dermatitis

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SUMMARY

Significantly elevated activity of peripheral blood mononuclear cell cAMP specific phosphodiesterase (PDE) activity has been previously reported in adults with atopic dermatitis and in the cord blood of children born to atopic parents. We have been unable to confirm such a significant elevation in children with atopic dermatitis, and we have not found any correlation with dermatitis severity, age or total serum IgE.

After Szentivanyi¹ first proposed his theory of partial β adrenoreceptor blockade to account for many of the pharmacological and immunological abnormalities noted in atopic disorders, several investigators studied the response of the intracellular secondary messenger cyclic 3'5' adenosine monophosphate (cAMP) to β adrenergic agonist stimulation. When it was discovered that the blunted elevation in cAMP noted after β agonist stimulation of atopic mononuclear cells² was also evident after histamine and E series prostaglandins stimulation,³ attention focused on biochemical events which lay distal to the β adrenoreceptor.

Grewe, Chan and Hanifin⁴ first documented elevated activity of cAMP specific phosphodiesterase (PDE) in peripheral blood leukocytes in adult patients with atopic dermatitis (AD). This enzyme catalyses the hydrolysis of cAMP to 5' adenosine monophosphate; thus high activity of PDE may diminish the rise in cAMP seen in normal leukocytes after β agonist and histamine stimulation.

The finding of elevated PDE activity in the cord blood of children born to atopic parents,⁵ the demonstration of high PDE activity in non eczematous atopic individuals with allergic rhinitis, and the finding of elevated PDE activity after remission of atopic dermatitis in adults⁴ has led to the supposition that elevated PDE activity may be a primary biochemical abnormality of aetiopathological significance to the atopic state. The finding of normal PDE activity in

widespread contact dermatitis⁴ provides evidence that elevated PDE activity may be specific to the atopic state.

Atopic dermatitis is more commonly a disease that affects children, and to date studies have only reported on PDE activity in adult atopics. According to one large series⁶ only about 10% of children with AD continue to have active disease until adult life, this group may represent a severe and resistant subset of atopic dermatitis sufferers. We have determined PDE activity in peripheral blood MNLs in a group of children with atopic dermatitis of varying severity, and compared this to MNL PDE in non eczematous children. We attempted to determine if there was any correlation between PDE activity and the severity of eczema and other clinical parameters and total serum IgE concentration.

METHODS

This research project was approved by the Hospital Ethical Committee and children with atopic dermatitis varying in severity but fulfilling the Hanifin-Rajka criteria were recruited from the skin clinics of St. Helier and St. George's Hospitals and from amongst members of the local branch of the National Eczema Society. Due to the difficulty of making a definite diagnosis of atopic dermatitis in early infancy, children over the age of 2 years and below the age of 12 years were recruited. Non-eczematous children in the same age range prior to routine admission of ENT surgery were selected for comparison and these controls had been free of infective exacerbations of their ENT conditions (tonsillitis, sinusitis and middle ear infections) for at least 2 weeks. Caucasian, Afro-Caribbean and Asian children were included in the study. In all cases a detailed history of subject and family history of atopic manifestations was obtained from the parents. All children were examined for the presence of eczema. On the basis of this, children were allocated to the following groups:

Group 1. Atopic dermatitis fulfilling the Hanifin-Rajka criteria.⁷

Group 2. Controls (no personal or first degree relatives with a history of any atopic manifestations, e.g. asthma, atopic dermatitis, allergic rhinitis).

Group 3. Controls with a first degree atopic relative (the subjects having no personal, but having a first degree relative with a history of an atopic disease).

Group 4. Non-eczematous atopics. Children with asthma or hayfever without a past history of eczema or eczema at the time of examination prior to venepuncture.

The eczema area activity score was determined by a single observer (IHC) using the method described by Neild *et al.*⁸ for all of the children with atopic dermatitis.

Ten millilitres of peripheral venous blood was obtained from all subjects between 12:00 and 14:00 for MNL PDE activity estimation. All subjects had avoided caffeine containing beverages (tea, coffee, cola and cocoa) for at least 12 h before venepuncture. No subject had taken any oral medication for at least 5 days, and emollients only had been used for a week before blood sampling. In the most anxious children, prilocaine and lignocaine local anaesthetic cream (EMLA cream, Astra Pharmaceuticals) was applied to the venepuncture site under an occlusive dressing (Tegaderm, 3M) for 40 min before venepuncture.

MNL separation

In all cases MNL separation was undertaken within 4 h of venepuncture. Five millilitres of heparinized blood was diluted with an equal volume of phosphate buffered saline (PBS) and MNL separated by Hypaque-Ficoll centrifugation. The cells were washed 3 times in PBS EDTA, and centrifuged at 350, 300 and 250 g to remove platelets. After counting using a

haemocytometer and methylene blue dye to ensure there were no platelet or erythrocyte contamination, the cells were suspended at 2 or 4×10^6 cells/ml in Grey's Balanced Salt solution (GBSS) and stored frozen at -70°C until required for PDE assay.

cAMP phosphodiesterase assay

Thawed samples were sonicated for 25 s at 4°C using an Ultrasonic Rapidis 180 sonicator and diluted to 1×10^6 cells/ml with GBSS. 200 μl of the diluted homogenate were then added to 200 μl of cAMP substrate ($0.05 \mu\text{M}$, 200 μl) containing 20,000 cpm of (^3H) cAMP (Amersham International, Amersham) in 40 mM Tris HCl buffer (pH 8.0) containing 3.75 mM β mercaptoethanol and 50 mM MgCl_2 .

Previous kinetic studies by ourselves and others in adults have shown a greater discrimination between atopic and non atopic individuals' MNL PDE activities at low substrate concentrations^{4,9}; $0.05 \mu\text{M}$ cAMP concentration gave the optimal distinction between these two groups. Blank samples contained 200 μl of GBSS and 200 μl of cAMP substrate. The samples were incubated at 30°C for 10 min and then transferred to a boiling waterbath for 90 s, cooled and frozen. Having returned the reaction mixtures to 4°C , 0.45 units/ml of $5'$ nucleotidase in 40 mM Tris HCl buffer (pH 8.0) was added and then incubated at 30°C for 10 min. The tritiated adenosine was separated from the reaction mixture using 0.7 ml Dowex $1 \times 2-400 \text{ Cl}^-$ anion exchange column (applied as 1.4 ml of a 50% v/v slurry in distilled water). The radioactive adenosine was eluted with 1 ml + 2×2.5 ml of distilled water. 0.5 ml of the eluent was counted in 3 ml of scintillation fluid using a LKB 1212 Rackbeta liquid scintillation counter. Having determined the protein present in each sample using the method of Lowry *et al.*,¹⁰ the PDE activity was expressed as pmol cAMP hydrolyzed /min/mg protein. Total serum IgE was assayed using the Phadebas IgE PRIST® kit (Pharmacia G.B. Ltd, Milton Keynes, Bucks, U.K.).

Results are expressed as the mean $\pm$ the standard error of the mean, and the Mann-Whitney *U*-test was used to determine statistical significance.

RESULTS

Results of the peripheral blood MNL PDE activity estimations are shown in the form of a scattergram in Figure 1, with statistical analysis of the data in Table 1. There was no statistical difference between the PDE activities for the atopic dermatitis patients (group 1) compared to the control children (group 2), the children with first degree relatives with an atopic history (group 3) and non-eczematous atopic children (group 4).

In the atopic dermatitis group, there was no correlation between the atopic dermatitis area activity score and the PDE activity ($r = 0.177$), or the PDE activity and the total serum IgE ($r = -0.2$) and age ($r = 0.156$). There was only a weak positive correlation between age of onset of eczema and PDE activity ($r = 0.40$). Mean PDE activity was highest in the atopic children with only eczema, and was lowest in those with eczema asthma and hayfever, but differences did not reach statistical significance at the $P < 0.05$ level (Table 2).

DISCUSSION

Since the report of Grewe, Chan and Hanifin⁴ that leukocyte PDE activity is elevated in adult atopic individuals, particularly those with atopic dermatitis, several other investigators, including ourselves,⁹ have confirmed this result and the elevated activity has been localized to the monocyte subset of peripheral blood leukocytes.¹¹ Grewe, Chan and Hanifin found a clear

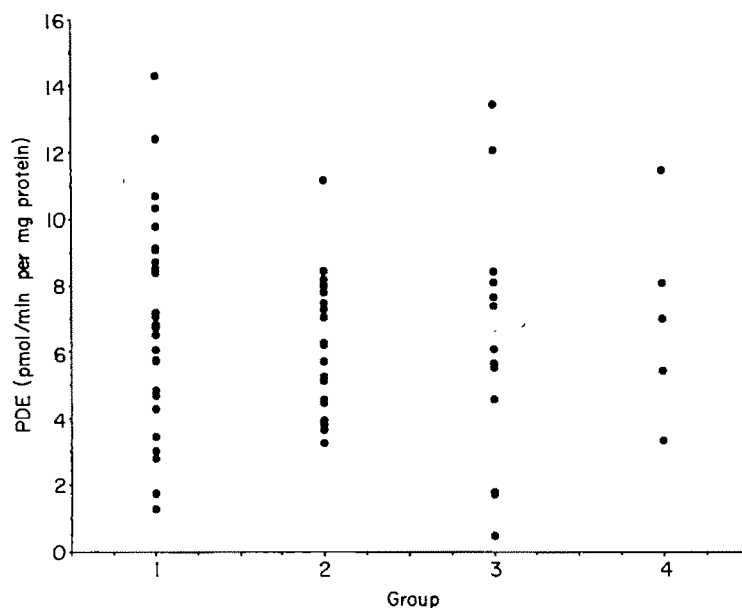


FIGURE 1. Scattergram of PDE activity. Group 1, AD sufferers; group 2, control children without a personal or first degree relative with any atopic manifestation; group 3, controls without a personal history of an atopic manifestation, but with an atopic first degree relative; group 4, non eczematous atopic children.

separation between the non-atopic control population and the atopics, with the enzyme activity in the atopic MNLs being greater than two standard deviations above the mean of controls. In addition they found that three patients in remission from their atopic dermatitis also had elevated MNL PDE activity, and as a result, they suggested that elevated PDE activity may reflect a primary biochemical abnormality of atopy. The finding that normal non-atopic MNL

TABLE 1. MNL PDE activity in the groups defined in the patients section. All results expressed as mean $\pm$ SEM *P* is significance level using Mann-Whitney *U*-test

Group	Number	Age years	MNL PDE activity pmol/min per mg protein	<i>P</i>
1	29	5.7 $\pm$ 1.4	6.71 $\pm$ 0.58	
2	21	5.6 $\pm$ 1.6	6.34 $\pm$ 0.44	0.49
3	13	7.5 $\pm$ 1.4	6.34 $\pm$ 1.05	0.49
4	5	7.1 $\pm$ 1.5	7.01 $\pm$ 1.35	0.47

Group 1, AD sufferers; group 2, control children without a personal or first degree relative with any atopic manifestation; group 3, controls without a personal history of an atopic manifestation, but with an atopic first degree relative; group 4, non-eczematous atopic children.

TABLE 2. PDE activity in the dermatitis sufferers (group 1) listed according to other atopic manifestations (mean $\pm$ SEM)

Atopic status	n	MNL PDE activity (pmol/min per mg protein)
Eczema only	12	8.23 $\pm$ 0.92
Eczema and hayfever	2	7.26
Eczema and asthma	7	5.59 $\pm$ 1.17
Eczema, asthma and hayfever	8	5.27 $\pm$ 0.92

PDE activity is elevated by incubation with histamine¹² however, raises the point that elevated PDE activity in atopy may be secondary to mediators of cutaneous inflammation.

Our results have failed to demonstrate the differences between MNL PDE activity in children with atopic dermatitis and normal children, children with a family history of atopy, and non-eczematous atopic children that we⁹ and others have shown in equivalent adult groups. PDE activity values found in children are similar to those of non-atopic adults reported from this laboratory. One possible explanation for this is that we have submitted children to venepuncture between 12:00 and 14:00, whereas in all of the previous studies, adults have been bled early in the morning. As yet it is not known whether MNL PDE undergoes diurnal variation. As in the adults, we have failed to demonstrate any correlation between PDE activity and eczema activity and total serum IgE. The weak positive correlation between age of onset and PDE activity is of interest. Vickers⁶ has highlighted late age of onset of atopic dermatitis as a poor prognostic sign associated with chronicity and persistence of eczema into adult life. One possible way of reconciling our results of childhood PDE with the significantly elevated PDE activity in adult atopics may be that only those children with the highest PDE activities and late onset eczema continue to have eczema into adult life, whereas the early onset eczema sufferers have lower PDE activities and they undergo spontaneous remission of their dermatitis. Prolonged follow up with sequential PDE estimations will be required to see if this is the case and will help to determine if PDE estimation in childhood will be of value in predicting long-term prognosis.

Heskel *et al.*⁵ have determined umbilical cord blood MNL PDE activity and demonstrated higher activity in infants born to atopic parents, regardless of whether they had an atopic mother, an atopic father or both, compared to those born to non-atopic parents. Limited follow up for 6 months suggested that infants born with a high cord blood PDE to atopic parents had an increased incidence of atopic dermatitis and associated features. Results of longer term follow up of these infants and subsequent PDE activities have not been reported. These cord blood studies and our own childhood PDE results would appear to be contradictory. However, it is probable that most of the infants delivered would have been exposed to opiate drugs administered to their mothers during labour only hours before collection of the cord blood samples. Opiates cross the placenta with ease and are well known to effect direct mast cell histamine release.¹³ It may be that opiate-induced histamine release may be more pronounced in atopic mothers or their infants; indeed an increased histamine 'releasability' has been documented in atopic adults.¹⁴ It is well established that histamine *in vitro* will activate PDE¹² and this perhaps may be more pronounced in the atopic children. Also neonatal blood normally

contains nucleated erythrocytes which because of their mass may be expected to centrifuge in a way similar to mononuclear leukocytes. Contamination of the mononuclear cell layer with erythrocytes may account for a further possible difference between the PDE results in the cord blood study compared to those determined in the older children reported here.

In summary, we have been unable to document an elevated MNL PDE activity in atopic dermatitis in children, who represent numerically the largest group of sufferers. It is possible that the blunted cAMP responses in MNLs to β adrenoreceptor agonists documented in adults with atopic diseases are not manifest in children suffering from the same diseases and the results of this study would indicate that this premise requires investigation in children too.

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Keratinocyte expression of OKM5 antigen in inflammatory cutaneous disease

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SUMMARY

Keratinocyte expression of the monocyte/macrophage surface antigens defined by OKM1 and OKM5 antibodies (Ortho Diagnostics) was examined using the peroxidase anti-peroxidase immunohistochemical technique. A range of inflammatory cutaneous disorders were investigated, including lichen planus, psoriasis and atopic dermatitis. Positive suprabasal keratinocyte expression of OKM5 antigen was observed in all disorders, while keratinocyte staining with OKM1 antibody was consistently negative. These results provide further evidence that keratinocytes may play an important role in cutaneous immune responses. Furthermore, they are consistent with the recent observation that HLA-DR positive keratinocytes may modulate cutaneous immunological reactions by inducing T-cell unresponsiveness.

Monoclonal antibodies OKM1 and OKM5 define two distinct antigens of molecular weights 170 and 80 kDa respectively, present on the surface of the majority of peripheral blood monocytes.¹ OKM1 antigen, which has been identified further as the receptor for the C3bi complement component,² is also present on granulocytes and on null cells.³ OKM5 antigen, on the other hand, is expressed by monocytes, platelets¹ and some endothelial cells,⁴ but its precise function has yet to be determined. It appears to be important in the accessory cell function of monocytes since only OKM5 positive cells can stimulate the autologous mixed lymphocyte reaction.⁵

In normal human epidermis, keratinocytes and Langerhans cells do not express either OKM1 or OKM5 antigen. Recent studies, however, have shown that keratinocytes from positive Mantoux reactions, a model of cell-mediated immunity, express OKM5 antigen.⁶ Furthermore, expression of this antigen by human keratinocytes, *in vitro*, can be conferred by the activity of interferon-gamma (IFN- γ),⁷ a cytokine derived from activated T lymphocytes. Since the predominant cell type of the dermal infiltrate in most inflammatory cutaneous diseases is the

helper T lymphocyte, many of which are activated,⁸ OKM5 expression by keratinocytes in these disorders might be anticipated.

We have, therefore, undertaken an immunohistochemical study to determine if keratinocytes *in vivo* express the monocyte markers OKM1 and OKM5 in inflammatory cutaneous diseases. If present, their expression may indicate an important role for keratinocytes in cutaneous immune responses.

METHODS

Skin biopsies were obtained from consenting adult patients with the following diseases: lichen planus (LP) ($n=8$), discoid lupus erythematosus (DLE) ($n=5$), atopic dermatitis (AD) ($n=6$), psoriasis ($n=10$) and cutaneous T-cell lymphoma ($n=1$). Biopsies were also obtained from positive experimental nickel sulphate patch test reactions at 48 h ($n=3$) and 96 h ($n=3$) and from normal skin ($n=5$). All material was embedded in OCT, snap frozen in a bath of isopentane, cooled in liquid nitrogen and stored at -190°C .

Subsequently 5 μm sections were cut from each biopsy, air-dried on glass slides and fixed in acetone for 10 min prior to the addition of the primary monoclonal antibody as listed in Table 1. A three-step peroxidase anti-peroxidase method was used, as described elsewhere⁹ using diaminobenzidine (DAB) as the substrate. The slides were counterstained with haematoxylin, dehydrated, and mounted, positive labelling being identified as a distinctive brown reaction product visible at the light microscope level. Two negative controls were included: firstly, omission of the primary monoclonal antibody and, secondly, substitution of the primary antibody with an irrelevant antibody of the same immunoglobulin subclass.

RESULTS

In normal skin staining with OKM5 antibody was observed on scattered cells, presumably macrophages, within the dermis, and on cells of the acrosyringium. Endothelial cells, keratinocytes and Langerhans cells (OKT6 +ve, HLA-DR +ve) did not express OKM5 antigen (Fig. 1). OKM1 was observed only on a few dermal cells.

Keratinocytes, overlying the dermal inflammatory infiltrate, were consistently positive for OKM5 antigen in all disorders studied (Fig. 2), including psoriasis (Fig. 3) and atopic dermatitis where keratinocyte HLA-DR expression was rarely seen. Keratinocytes were uniformly negative for OKM1, but both OKM1 and OKM5 antibodies stained cells within the dermal infiltrate. OKM5 staining was maximal in the prickle cell and granular cell layers. In lichen planus and discoid lupus erythematosus this pattern of OKM5 staining was different from that observed with HLA-DR, where keratinocyte expression was visible throughout the

TABLE 1. Primary monoclonal antibodies (McAb) used in study

McAb	Cluster determinant	Specificity	Source
OKM5	CD36	Monocyte/platelet	Ortho
OKM1	CD11b	C3bi receptor	Ortho
L243	—	HLA-DR	Becton-Dickinson
OKT6	CD1	Langerhans cells/thymocytes	Ortho

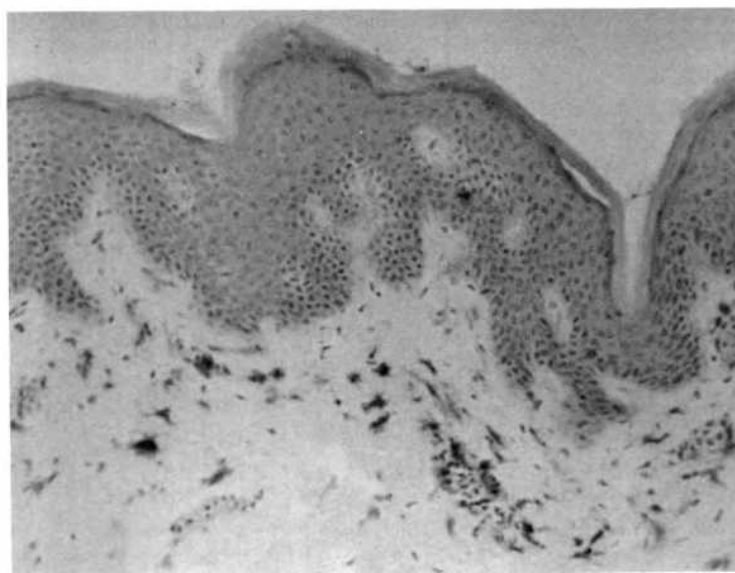


FIGURE 1. Photomicrograph of normal skin stained with OKM5 antibody. Scattered cells within the dermis stain positively, but the epidermis is uniformly negative. (Original $\times 100$).

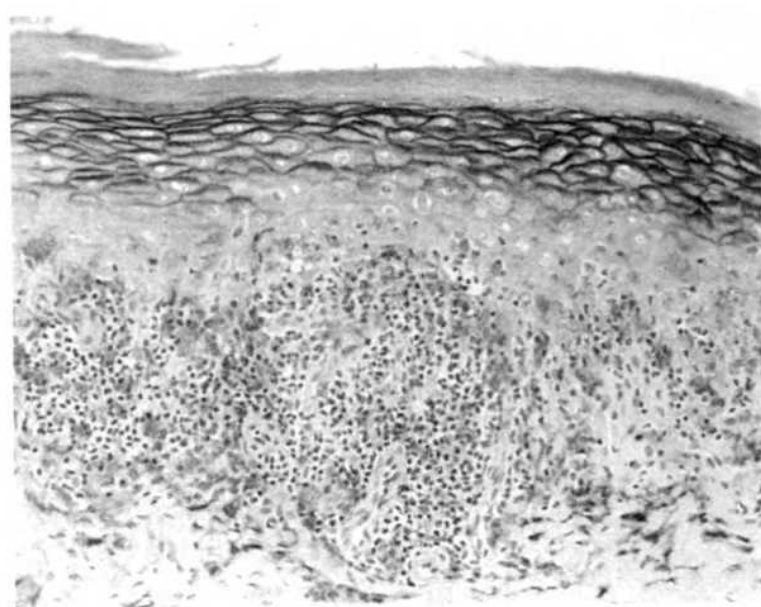


FIGURE 2. Photomicrograph of lichen planus stained with OKM5 antibody. Strong suprabasal keratinocyte expression can be observed overlying the dermal infiltrate. (Original $\times 120$).

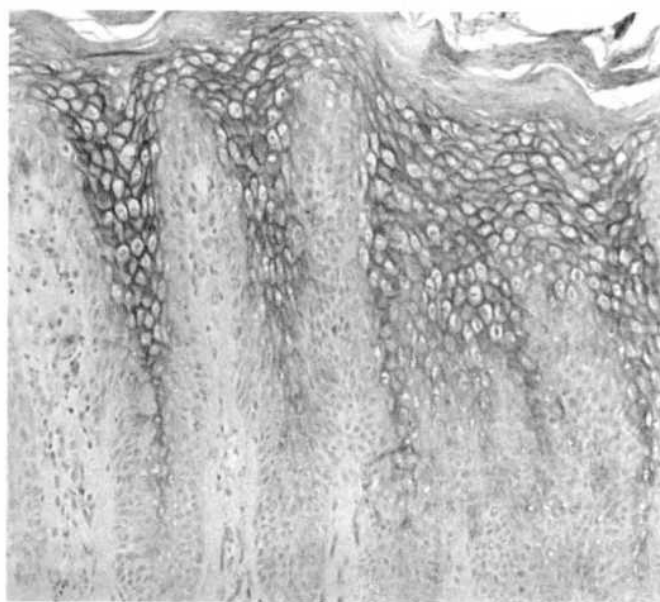


FIGURE 3. Photomicrograph of lesional psoriasis stained with OKM5 antibody. Suprabasal keratinocyte expression is prominent, particularly in the epidermis overlying the elongated rete ridges. (Original $\times 105$).

TABLE 2. Number of patients with positive keratinocyte staining for each primary McAb

Disease	Number of			
	patients	DR + ve	M5 + ve	M1 + ve
Lichen planus	8	7	8	1
DLE	5	5	5	0
Psoriasis	10	2	10	0
Atopic dermatitis	6	0	6	0
CD (48 h)	3	0	0	0
CD (96 h)	3	2	3	0
CTCL (Plaque stage)	1	1	1	0
Normal skin	5	0	0	0

full thickness of the epidermis. Furthermore, staining with OKM5 compared with HLA-DR was more intense, and in the one biopsy of lichen planus where HLA-DR was absent from keratinocytes, OKM5 was present. Weak positive keratinocyte staining with OKM5 was seen in allergic contact dermatitis at 96 h, while staining was absent at 48 h, similar to the results obtained with HLA-DR.

The full results are tabulated in Table 2.

DISCUSSION

This study demonstrates that, in the conditions examined, keratinocytes express OKM5 antigen, but not OKM1 antigen. Furthermore, expression appears to be limited to those

keratinocytes overlying the dermal infiltrate, consistent with induction of keratinocyte OKM5 antigen synthesis by IFN- γ . The possibility that keratinocytes acquire shed OKM5 antigen from dermal leukocytes cannot, however, be excluded.

The majority of peripheral blood monocytes express both OKM1 and OKM5 antigens together with HLA-DR on their cell surface. These cells are able to present soluble antigens to T lymphocytes,¹⁰ thus inducing T-cell proliferation. However, a small subgroup of monocytes lack OKM1 antigen, while expressing OKM5 antigen (HLA-DR+, OKM1-, OKM5+).¹ These cells, in addition to presenting soluble antigen, are also able to activate autoreactive T lymphocytes in the absence of added antigens,⁵ a function which can be assessed via the autologous mixed lymphocyte reaction. Keratinocytes from many inflammatory cutaneous disorders share several immunophenotypic similarities with this subgroup of monocytes. They are HLA-DR positive,¹¹ synthesize interleukin-1¹² and, as we report here, express OKM5 antigen, but lack OKM1 antigen. This suggests that keratinocytes may be able to serve similar immunological functions to this subgroup of monocytes, as has been postulated for vascular endothelial cells.⁴

To date, the precise function of HLA-DR positive keratinocytes remains unknown. Although they can serve as targets for HLA-DR restricted cytotoxic T cells,¹³ they are unable to present antigen to helper T cells in the allogeneic epidermal cell-lymphocyte reaction.¹⁴ However, recent evidence from Gaspari *et al.* suggests that HLA-DR positive keratinocytes may modulate cutaneous immunological reactions by inducing T-cell unresponsiveness to specific antigens.¹⁵ This hypothesis is further supported by our observation that keratinocytes from inflammatory disease express OKM5 antigen, but not OKM1 antigen, since activation of the autologous mixed lymphocyte reaction leads to suppression of many lymphocyte-mediated responses.¹⁶

OKM5 antigen expression by keratinocytes has previously been described in positive Mantoux reactions,⁶ and in plaque stage cutaneous T-cell lymphoma.¹⁷ Taken together with our findings, this suggests that OKM5 positivity may be expected in any condition characterized by a predominantly T lymphocyte dermal infiltrate. It is of interest that OKM5 antigen was present on keratinocytes in both atopic dermatitis and psoriasis where keratinocytes rarely express HLA-DR, despite the presence of a dermal infiltrate containing activated T lymphocytes.^{18,19}

The identification of the macrophage/monocyte antigen OKM5 on the surface of keratinocytes increases the number of immune function associated antigens observed on these cells. This provides further evidence that keratinocytes may play an important role in cutaneous immune responses.

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Calcitonin gene-related peptide immunoreactivity in prurigo nodularis: a comparative study with neurodermatitis circumscripta

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SUMMARY

Eight patients with intensely pruritic lesions of chronic idiopathic prurigo nodularis and three patients with neurodermatitis circumscripta were investigated using the indirect immunofluorescence method. Results showed similarities in epidermal hyperplasia but not in nerve proliferation and neuropeptide immunoreactivity. Increased numbers of calcitonin gene-related peptide (CGRP) and substance P immunoreactive nerve fibre bundles were detected in specimens taken from prurigo nodularis lesions, but no increased immunoreactivity could be seen in specimens taken from patients having neurodermatitis circumscripta compared to normal skin. The neuropeptides, CGRP and substance P, may be responsible for the intense itching of prurigo nodularis lesions.

Patients having chronic nodular skin lesions with itching were described by Hardaway in 1880.¹ The same eruption was named prurigo nodularis by Hyde in 1909.² Lichen corneus obtusus has also been assumed to represent the same condition.³ Prurigo nodularis is often classified as an atypical nodular form of neurodermatitis circumscripta.^{4,5} The aetiology of the condition is unknown. Emotional factors, local trauma, insect bites, gluten enteropathy and other metabolic disturbances have been proposed as at least contributory factors.³⁻⁸

Prurigo nodularis is mainly a clinical diagnosis. Dome-shaped, intensely pruritic nodules are located symmetrically on the extensor sites of the extremities and occasionally on the trunk. The histopathological features of prurigo nodularis resemble those of neurodermatitis circumscripta, although differences exist.⁹ Many investigators have described nerve fibre proliferation in

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prurigo nodularis lesions¹⁰⁻¹⁵ but contradictory views have also been reported.⁸ This study was performed to investigate neural proliferation and the occurrence of neuropeptides in prurigo nodularis and neurodermatitis circumscripta. In the present paper we describe our findings at light microscopic level and discuss possible connections between neuropeptides and pruritus.

METHODS

Skin biopsies were obtained from eight patients having clinically typical, chronic prurigo nodularis (four males, four females) and three patients having neurodermatitis circumscripta (one male, two females). Biopsies were also studied from volunteers without any history of skin disease. The biopsy specimens were taken under local anesthesia (Lidocain®, Orion, Finland), immersed in 4% paraformaldehyde containing 0.3% picric acid and fixed at 4°C for 2-4 h. They were then rinsed overnight in phosphate buffered saline (PBS). The indirect immunofluorescence method was used to demonstrate immunocytochemically neuropeptides in cutaneous nerves. Frozen sections, 14 µm thick, were incubated first with a 1:400-1:800 dilution of the primary antibody (raised in rabbits, see below) for 48 h at 4°C, and then with a 1:80 dilution of tetramethyl rhodamine-isothiocyanate (TRITC)-conjugated goat anti-rabbit antibody (Boehringer Mannheim Biochemicals, West Germany) for 30 min at 37°C. Between different steps the sections were rinsed for 15 min in PBS. All antisera were diluted in PBS containing 0.3% Triton X-100. For control sections, the primary antibodies were pre-absorbed with pure peptide (50 µg peptide/ml diluted antiserum) respectively.

The antisera used were against somatostatin (SOM), natural porcine vasoactive intestinal polypeptide (VIP), natural porcine peptide histidine-isoleucine (PHI) (Milab, Malmö, Sweden), synthetic porcine galanin, synthetic porcine neuropeptide Y (NPY), synthetic rat calcitonin gene-related peptide (CGRP) and substance P (Peninsula Laboratories, Belmont, NY, U.S.A.) Some sections from each specimen were stained with haematoxylin and eosin for routine histologic examination.

RESULTS

Prurigo nodularis

The number of CGRP immunoreactive nerves in the lesions of prurigo nodularis patients was markedly increased as compared to the surrounding uninvolved skin or healthy volunteer's skin (Figs 1-3). CGRP immunoreactivity was seen in thick preterminal nerve bundles in the dermis (Fig. 3) extending and ramifying in thin varicose nerve fibres beneath the epidermis (Fig. 2). The downward protruding epidermal rete ridges, in particular, were surrounded by numerous CGRP immunoreactive nerve fibres (Fig. 2). The bulk of the nerves was seen at the centre of the nodules. The peripheral parts of the nodules contained less CGRP nerves. CGRP immunoreactive nerve fibres could also be detected around blood vessels and sweat glands, but the density did not differ from that of healthy skin. The findings were similar in each specimen and there seemed to be no differences between sexes. The number of substance P immunoreactive nerves was higher in prurigo nodularis lesions than in healthy skin, but the difference was not as conspicuous as with CGRP.

Neurodermatitis circumscripta

The number of CGRP and substance P immunoreactive nerves did not differ from that of healthy skin. The only similarity with prurigo nodularis specimens was epidermal hyperplasia and hyperkeratosis.



FIGURE 1. Immunofluorescent labelling of healthy human skin showing thin varicose CGRP immunoreactive nerve fibres beneath the epidermis. In the dermis CGRP immunoreactive nerves are sparse. Bar 20 μm .

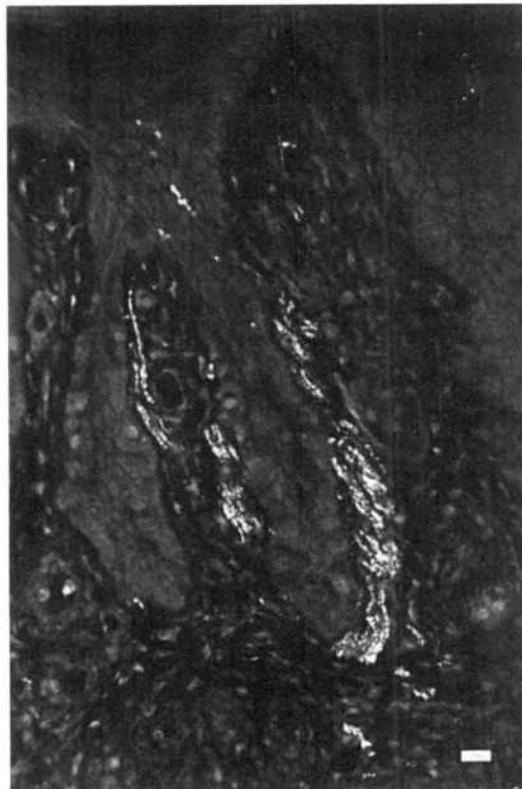


FIGURE 2. Fluorescent labelling of numerous subepidermal CGRP immunoreactive nerve bundles in the lesion of a prurigo nodularis patient. Bar 20 μm .

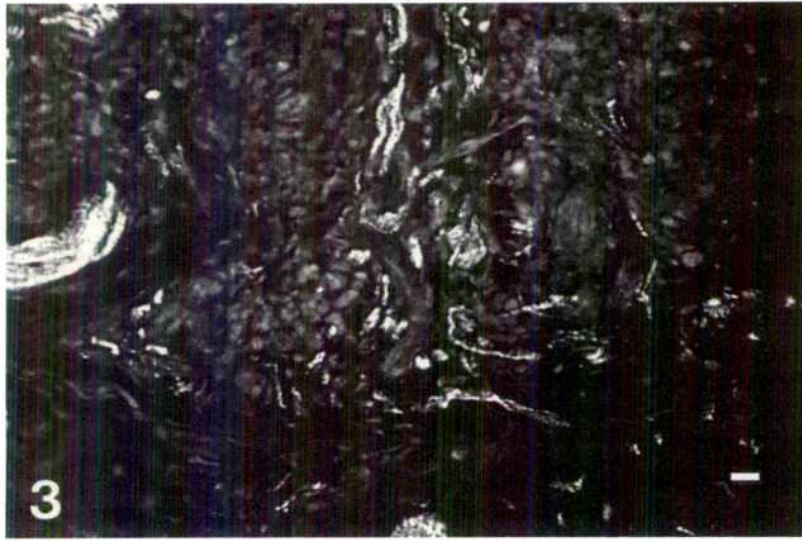


FIGURE 3. Immunofluorescent labelling of the upper dermis of the lesion of a prurigo nodularis patient. The number of CGRP immunoreactive nerve fibres is considerably higher compared to healthy skin in Figure 1. Bar 20 μ m.

The number of nerves showing immunoreactivity to VIP, PHI, SOM, NPY or galanin in either prurigo nodularis lesions or neurodermatitis circumscripta did not differ from that of normal skin.

DISCUSSION

The sensory nerves in normal human skin show immunoreactivity against CGRP and substance P, although the innervation varies in different areas of the body.¹⁶ The present results show that CGRP and substance P immunoreactive nerves in the prurigo nodularis lesions were markedly increased in number compared to the normal human skin or the uninvolved skin of prurigo nodularis patients. It has been suggested that prurigo nodularis is an atypical form of neurodermatitis circumscripta. It is worth noticing that despite such views, we could not demonstrate increased neuropeptide immunoreactivities in specimens taken from neurodermatitis patients.

It is still unknown which occurs first in prurigo nodularis patients, itching or scratching. Is the nerve proliferation due to scratching, or could there be another agent that causes nerve proliferation that leads to a vicious circle of itching and scratching? It has been suggested that mechanical trauma could lead to proliferation of peripheral nerves.¹⁷ Obviously scratching can cause damage in cutaneous nerves and stimulate nerve proliferation.¹⁴

Specific neuropeptide staining patterns have been found in non-neuronal cells in certain skin diseases such as urticaria pigmentosa, lichen sclerosus et atrophicus, diabetic lipodystrophy, bullous pemphigoid and other disorders.⁶ Involvement of substance P containing sensory nerves has been suggested in cold and heat urticaria.¹⁸ The role of neuropeptides in these skin diseases is not clear. In normal human skin neuropeptides are likely to be mediators of neurogenic inflammation and thus possible mediators of allergic and inflammatory reactions of the skin.¹⁹ Some neuropeptides, like substance P and CGRP, are potent histamine-releasing

agents in man.²⁰⁻²³ Substance P and CGRP could be responsible for the severe pruritus of prurigo nodularis lesions either directly or indirectly by liberating histamine from mast cells.

Prurigo nodularis patients have been treated with topical steroids, photochemotherapy, cryotherapy, antihistamines, etc. Bearing in mind the present results, a new therapy for these patients could be topical capsaicin. Capsaicin is able to induce reversible functional impairment of substance P and CGRP containing primary sensory fibres²⁴ that convey both pain and itch sensations. Further studies are in progress to investigate further the relationship between neural proliferation, neuropeptides and itching.

ACKNOWLEDGMENT

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Human cutaneous mast cells—a study of fixative and staining reactions in normal skin

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SUMMARY

Routinely used formal saline fixation reduces the number of demonstrable mast cells in human skin by up to 30% compared with paired specimens fixed in Carnoys medium. Using metachromatic (toluidene blue), orthochromatic (alcian blue/safranin), enzymatic (chloroacetate esterase reaction) and immunofluorescence (berberine and fluorescein conjugated avidin) staining techniques, mast cells were demonstrated and quantified. Alcian blue/safranin and fluorescein-conjugated avidin were both superior to the other staining methods used. We recommend the use of Carnoys medium fixed tissue stained with either alcian blue/safranin or conjugated avidin for optimal visualization and assessment of mast cells in human skin.

To assess mast cells objectively in histological specimens of skin it is important to establish the optimum method for their preservation and demonstration. It is well established that certain fixatives impair demonstration of mast cell granules in several species. This is exemplified by rat mast cells in the lamina propria of the intestine, so called mucosal mast cells (MMC), which are exquisitely sensitive to formalin, necessitating the use of other fixatives such as Carnoys medium for their demonstration.¹ Mast cells from rodent peritoneal cavity and skin, so called connective tissue mast cells (CTMC), by contrast are relatively formalin resistant.¹

The most characteristic feature of mast cells is based on the presence of dense metachromasia of their granules with basic thiazine dyes such as toluidene blue (TOL). However, other methods of staining have been used and may render additional information. The orthochromatic copper phthalocyanin dye, alcian blue, used in a staining sequence with safranin O (AB/SAF) has been used to stain differentially populations of rodent mast cells, MMC granules staining blue whereas CTMC granules stain a mixture of blue and red or just red.² The cellular enzyme chloroacetate esterase is present in mast cells and is stable during paraffin processing. Leder utilized this fact in developing an enzymatic technique for demonstrating

mast cells in tissue sections—the naphthol AS-D chloroacetate esterase method (C-EST).³ The fluorescent dye, berberine sulphate (BERB) has been found to bind with rodent mast cell heparin in cell suspension⁴ giving positive fluorescence with rodent CTMC but not with MMC.⁵ More recently, the glycoprotein avidin conjugated with the fluorochrome dye fluorescein isothiocyanate (FITC-AV) has been shown to bind specifically to rat, mouse and human mast cell granules.^{6,7}

We used the above metachromatic, orthochromatic, enzymatic and fluorescence methods of demonstrating mast cells on human cutaneous tissue which had been frozen or fixed in 10% formal saline or Carnoys medium. We undertook such a study to establish which combination of fixation and staining would best preserve mast cells with respect to numbers, granule staining and cell structure in normal human skin.

METHODS

Subjects

Fifteen subjects (nine male, six female) undergoing diagnostic biopsy for localized benign and malignant skin lesions gave informed consent for an additional biopsy to be taken from a distant uninvolved area of skin of the upper arm or abdomen for use in our study. Subsequent pathological examination of the presenting lesions confirmed their localized nature; five benign melanocytic naevi, four actinic keratoses, four basal cell carcinomas, two squamous papillomas.

Tissue preparation

Field anaesthesia using 2% lignocaine (without adrenaline) was performed and an elliptical biopsy removed with minimal trauma. A small specimen was taken from the apex of the ellipse, mounted in OCT (Tissue TEC, Miles, TX, U.S.A.) and snap frozen in liquid nitrogen. The remaining, majority of the biopsy was bisected—one half being placed in 10% formal saline, the other in Carnoys medium (see below). Tissue was left in fixative for 24 h prior to routine paraffin processing and embedding. Bisected edges were embedded uppermost allowing as close a comparison between initial 5 μ m sections from paired blocks as possible. Serial 5 μ m cryostat sections were also prepared. All sections were then stained with toluidene blue, alcian blue/safranin, chloroacetate esterase, berberine and FITC-Avidin.

Materials

(i) Fixatives

Carnoys medium—60 ml absolute ethanol, 30 ml chloroform, 10 ml glacial acetic acid.

10% Formal saline—90 ml 0.85% sodium chloride, 10 ml formaldehyde solution: final percentage formaldehyde 3.4–3.8%.

(ii) Staining reagents and techniques

TOLUIDENE BLUE—pH 0.5²

Toluidene blue powder (0.5 g) (Sigma Chemical Co., St. Louis, MO, U.S.A., Code Number 52040) was dissolved in 100 ml 0.5 N HCl (pH 0.5). Sections were stained for 30 min, rinsed in tap water for 2 min and dehydrated through graded alcohols, cleared in xylene and mounted in DPX (Gurr, BDN Ltd, Poole, Dorset, U.K.).

ALCIAN BLUE/SAFRANIN—pH 0.3²

Alcian blue powder (1.0 g) (Gurr, BDH Ltd., Code Number 74240) was dissolved in 100 ml of 0.7 N HCl. Concentrated HCl was added to lower the solution to pH 0.3. Sections were stained for 30 min followed by washing for 5 min in 0.7 N HCl. Sections were then counterstained using 0.5% safranin (Gurr, BDH Ltd., Code Number 50240) in 0.125 N HCl for 5 min, rinsed in tap water and dehydrated through graded alcohols, cleared in xylene and mounted in DPX.

CHLOROACETATE ESTERASE REACTION³**Reagents:**

- (1) 10 mg naphthol AS-D chloroacetate (Sigma Chemical Co., Code Number N-0758) in 1 ml N,N-dimethylformamide.
- (2) 20 mg sodium nitrite in 0.5 ml distilled water plus 0.5 ml of pararosaniline hydrochloride (4% solution in 2 N HCl).
- (3) Veronal acetate buffer pH 6.8.

Reagent 2 was added drop by drop to 30 ml of reagent 3 until the pH fell to 6.3. Reagent 1 was then added and the resulting mixture filtered. Sections were incubated in the clear red infiltrate for 1 h at 20°C. Sections were then washed in tap water and counterstained with Mayers haemalum for 10 min. After further washing in tap water, sections were dehydrated through graded alcohols, cleared in xylene and mounted in DPX.

BERBERINE SULPHATE⁴

Berberine sulphate (0.02 g) (Sigma Chemical Co., Code Number B-3376) was dissolved in 100 ml of redistilled ion free water. Sections were stained in the solution for 10 min and then washed for 5 min in redistilled water acidified with citric acid to pH 4, prior to mounting in glycerol and visualizing by fluorescence microscopy.

FLUORESCCEIN ISOTHIOCYANATE CONJUGATED AVIDIN⁶

Sections were washed for 10 min each three times in tris-buffered saline (TBS) prior to incubation with 1:200 dilution of FITC-avidin (Becton Dickinson, Oxford, U.K.) dissolved in TBS for 60 min at 20°C in a humidified chamber. Sections were then washed for 10 min each in TBS three times prior to mounting in glycerol and visualizing with fluorescence microscopy.

Controls

Rodent intestine, skin and human lung were used as positive controls to ensure that Carnoy's medium preserved MMC and to check that staining methods were producing results consistent with previously documented patterns where described.

Quantification

Mast cells were counted on a Zeiss photomicroscope III equipped for transmitted light and fluorescence, with $\times 40$ and $\times 100$ (oil immersion) objectives and a $\times 10$ eyepiece with counting graticule. Orientating the graticule along the dermo-epidermal junction, 10 sequential fields were counted using both $\times 100$ objective (to allow detailed examination of granule staining) and $\times 40$ objective (to offset the effect on results of local variations in mast cell density). Sections were counted without knowledge of the fixative used and counts expressed as mast cells per 10 relevant high power fields (hpf). All complete mast cell profiles were counted whether nuclei

were visualized or obscured. Eight non-serial sections of each fixative and staining combination were counted by one observer and the mean calculated and used in all statistical analysis.

Statistical analysis

Results are expressed as mean and standard error of the mean (SEM). Paired results between fixatives are compared using paired Student's *t*-test. Comparisons between different stains within each fixative were also analysed using Student's *t*-test. Duplicate counts on the same specimens were assessed by linear regression analysis to evaluate the reproducibility of the counting procedure.

RESULTS

Mast cells were clearly visualized in all specimens, except in those stained with berberine sulphate which failed to stain any human cutaneous mast cells. Absent staining was noted in both frozen and fixed tissue, despite the fact that rodent skin controls revealed strongly fluorescent mast cells with this dye.

In contrast to paraffin-embedded material, mast cell preservation was poor in all frozen sections; many cells were degranulated and the presence of large amounts of positively staining discharged granules made accurate cell counting impractical. However, the partially degranulated state of many of the mast cells allowed closer examination of the differential staining of the often obscured mast cell nucleus from its granules.

Staining patterns

In all Carnoys medium fixed specimens, mast cells were more sharply outlined, with better overall preservation of their shape and more intense staining of cytoplasmic granules than in paired formal saline fixed specimens. This was true for all the staining procedures used.

Alcian blue/safranin. This sequential staining gave vivid alcian blue positive granules overlying a pink mast cell nucleus. In formal saline fixed sections of two patients only, an occasional mast cell (less than 3% of total mast cells) was observed, seeming to contain both alcian blue and safranin staining granules. No such cells were seen in the Carnoys medium paired specimens. The use of alcian blue at pH 0.3 allows blue mast cells to stand out against a pale pink background, with little staining of surrounding tissues facilitating speed and accuracy of counting.

Toluidene blue. Using this stain at pH 0.5 overcomes the difficulty sometimes experienced in identifying densely metachromatic mast cells against a heavily stained background which can occur when using toluidene blue at pH 4. At pH 0.5 violet metachromasia stands out against a near absent background facilitating identification, but metachromasia was found to be highly variable not only at this low pH, but at levels intermediate between pH 0.5 and pH 4.

Chloroacetate esterase reaction. In both Carnoys medium and formal saline fixed specimens, this technique gave a brilliant red reaction product in the cytoplasm surrounding a basophilic nucleus. Partially degranulated mast cells in frozen tissue clearly showed the red reaction product to be staining individual granules.

FITC-conjugated avidin. This fluorescence technique gave excellent, reproducible results on

TABLE 1. Comparison of mast cell counts (expressed as mean per 10 hpf $\times 40$ objective + SEM) in Carnoys and formal saline fixatives using various staining techniques ($n=15$)

	AB/SAF	FITC-AV	TOL	C-EST
Carnoys	153 $\pm$ 16	128 $\pm$ 12	120 $\pm$ 14	93 $\pm$ 7
Formal saline	112 $\pm$ 13	130 $\pm$ 13	94 $\pm$ 12	66 $\pm$ 9
Mean % reduction	27%	0	22%	29%

frozen, formal saline and Carnoys medium fixed specimens, although fluorescence was considerably weaker and decayed faster in formal saline fixed specimens. Mast cells with brilliant green fluorescence which often masked cellular details stood out against minimal background autofluorescence.

Mast cell quantification

Duplicate counts on 10 specimens showed a correlation coefficient of $r=0.9$ ($P<0.001$) by linear regression analysis, confirming the internal reproducibility of the counting procedure.

In all 15 specimens, formalin fixation gave substantially reduced mast cell counts compared with Carnoys medium fixed tissue using alcian blue/safranin ($P<0.05$) and chloroacetate esterase ($P<0.001$). Toluidene blue showed a similar trend but did not reach significance. FITC-avidin was unique in showing no effect of fixation on mast cell counts (Table 1).

Substantial quantitative differences were also noted between the various staining techniques. Irrespective of which fixative was used, alcian blue/safranin and FITC-avidin gave substantially higher mast cell counts than either toluidene blue or chloroacetate esterase. Mean mast cell counts and mean percentage reductions using the various stains are shown in Table 1. In Carnoys medium which, in our study, maximized mast cell identification, alcian blue/safranin gave the highest mast cell counts, though when compared with FITC-avidin this did not reach statistical significance. Alcian blue/safranin gave significantly higher counts than both toluidene blue ($P<0.01$) and chloroacetate esterase ($P<0.005$). FITC-avidin gave significantly higher counts than chloroacetate esterase ($P<0.05$) but not toluidene blue. There was no significant difference between counts using toluidene blue and chloroacetate esterase. The superiority of alcian blue/safranin and FITC-avidin over toluidene blue and chloroacetate esterase, in demonstrating mast cells, was further substantiated in formal saline fixed tissue although overall mast cell counts were lower than the paired Carnoys medium values.

DISCUSSION

This study demonstrates that routinely used formal saline fixation of normal human skin reduces mast cell counts by 22–29% compared with paired specimens fixed in Carnoys medium when using toluidene blue, chloroacetate esterase or alcian blue/safranin as staining techniques. FITC-avidin was not affected by fixation in this study.

The phenomenon of formalin sensitivity of mast cells, first observed in rodents, was initially thought to be due to an inability of formaldehyde to precipitate mast cell granule acid mucopolysaccharides, a process considered necessary for subsequent binding of dyes,¹ the failure of mast cells to stain being due, therefore, to a dissolution of non-precipitated stainable material. Subsequent studies implicate the aldehyde moiety in precipitating a diffusion barrier

of protein, blocking the reaction of cationic dyes to dye binding groups on granule glycosaminoglycans. This barrier can be overcome by mild trypsinization or prolonging staining times to 3–5 days.⁵ Our figure of 22–29% ‘formalin sensitivity’ of human cutaneous mast cells is comparable to the 13–27% increase reported in toluidene blue positive mast cells in mastocytosis when staining times were increased from 30 min to 5 days.⁸

Carnoy's medium was a superior fixative to formal saline, both quantitatively in revealing substantially more mast cells, and qualitatively in better preservation of mast cell shape, and more intense staining of cytoplasmic granules. Carnoy's medium may facilitate dye binding due to its acidity, increasing ionic linkages between glycosaminoglycans and basic cationic dyes.⁹ Leder, however, suggested that all acid fixatives destroy the chloroacetate esterase enzyme.³ We encountered no such problems, and other groups have found Carnoy's medium-fixed human gut¹⁰ and nasal epithelium¹¹ to contain strongly positive chloroacetate esterase mast cells.

The wide variation in mast cell counts depending on the staining technique used has major implications. The characteristic metachromasia of mast cell granules, attributed to the binding of basic dyes to sulphated mucopolysaccharides via ionic linkages, has to date, formed the basis for defining the mast cell population. However, in our study such a metachromatic dye (toluidene blue), has demonstrated markedly reduced numbers of mast cells compared with alcian blue/safranin or FITC-avidin. Furthermore, the variability of metachromasia and marked sensitivity to dehydration further limits the reproducibility of this technique.

The full structure and staining mechanism of the copper phthalocyanin dye alcian blue is unknown.¹² It probably reacts with carboxylated and ‘O’ sulphated glycosaminoglycans whereas ‘N’ sulphated glycosaminoglycans preferentially stain with safranin.¹³ Using this staining combination with Carnoy's medium the highest number of mast cells in skin sections were revealed, and at pH 0.3 visualization and counting of mast cells was facilitated. Marshall *et al.*¹⁴ using alcian blue/safranin on human skin found variability in granule staining with 5% of mast cells not having solely alcian blue granules. Apart from two patients in our study whose formal saline- but not Carnoy's medium-fixed sections suggested some mixture of blue and red granules, all other sections contained alcian blue positive granules only. Using frozen sections (thereby removing fixative and embedding artefacts) only alcian blue granules were seen. Rodent intestine used as a positive control, however, clearly showed populations with blue and red granules. Chloroacetate esterase, like toluidene blue was very disappointing with respect to its low total mast cell counts. Wong *et al.*¹⁵ comparing chloroacetate esterase with toluidene blue found similar results using both methods. We agree with this finding but have shown that both these techniques are markedly inferior to alcian blue/safranin or FITC-avidin staining.

Berberine sulphate which satisfactorily stained rodent skin controls was completely negative on human skin. This negative finding is unexpected as human cutaneous mast cells do contain heparin and would, therefore, be expected to bind with berberine. Moreover, our control studies revealed small numbers of human lung mast cells staining positively with this technique, suggesting it may have applications for human tissue other than skin.

Bergstresser *et al.*⁶ described the binding of fluorochrome conjugated avidin to mast cells in mouse, other rodents and human skin, and not to other granulated cells in cutaneous tissue.⁷ We confirm the ease and reproducibility of this immunofluorescence technique which gave similar mast cell counts to alcian blue/safranin and significantly higher counts than toluidene blue ($P < 0.01$) and chloroacetate esterase ($P < 0.001$) in formal saline fixed tissue. A useful application of the ability to demonstrate mast cells in frozen tissue using this technique is double fluorescence staining using other cell markers such as immunoglobulin E on the same section.

The demonstration of formalin sensitivity by human cutaneous mast cells has similarities

with the rodent model. Indeed it was this phenomenon that first suggested the existence of two distinct populations of mast cells in the rat. However, in the rat, the finding of organs rich in one type of mast cell (for example, formalin sensitive) and devoid of the other (formalin resistant) together with marked staining differences between the two cell types mirroring the anatomical separation, strongly suggests a true dichotomy in the rodent population. Such a clear pattern was not observed in this study on human skin and interpretation of histochemical findings regarding possible mast cell heterogeneity must be guarded.

From this study two main points emerge: firstly, the routinely used fixative formal saline reduces mast cell numbers by approximately 25% and poorly preserves mast cell structure. Secondly, alcian blue/safranin and FITC-avidin are substantially superior staining techniques compared with toluidene blue or chloroacetate esterase—two stains which have formed the basis for many mast cell studies in the past. We, therefore, recommend the use of Carnoy's medium with either alcian blue/safranin or FITC-avidin stains for the assessment and demonstration of mast cells in human skin.

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Multiple infundibular tumours of the head and neck

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SUMMARY

A condition of multiple benign and sometimes self-healing infundibular tumours of the skin is described. It affects the sun-exposed areas of the head and neck in Caucasians. The individual lesions consist of a cluster of thick pockets, representing in many details the supra-sebaceous portions of the follicular unit. Follicular poroma, tumours of the deeper part of the follicular infundibulum, virus warts, inverted follicular keratosis and prurigo nodules do not correspond adequately with the appearances described in the present series of six cases.

Over the past 12 years, six individuals with a novel type of eruptive skin tumour have been observed. These cases resembled each other fairly closely, and differed as a group from other conditions. The abnormal elements in the tumours strongly resembled the supra-sebaceous tracts of hair follicles.

The patients were all Caucasian, middle-aged men, of different origins (German, Greek, British, South African), and with no family history of the lesions. The tumours were scattered in the sun-exposed parts of the head and neck and were mostly some 3–5 mm in diameter. They were round, vegetating and scabbed papules, although occasionally larger and more plaque-like. Some normal hair growth penetrated through them. The tumours in question continued to erupt for many years and there were always a few active lesions present. Some healing, either partial or complete, always occurred and often this followed treatment, but many were possibly destroyed by the patients, excoriating or plucking the lesions. The resulting scars were usually flat, broad, depressed and pale, or were curved or angulated and acneiform. The scarring seen suggested artefact or burnt-out discoid lupus erythematosus. Active lesions were sometimes placed at the periphery of such scars, or along an otherwise well-healed excision line.

CASE REPORTS

The data concerning these patients are shown in Table 1. The appearances of the lesions are shown in Figures 1a, b.

TABLE 1. Multiple tumours of the infundibular hair follicle unit

Case reference no.	1	2	3	4	5	6
Age at first visit	47	30	61	44	54	49
Sex	M	M	M	M	M	M
Country of origin	Germany	South African white	U.K.	Cyprus	South African white	South African white
Duration of complaint at first visit	5 years	4 months	'Lifelong'. Worse past 4 years	5 years	8 years	10 years
Length of follow-up after first visit	12 years	nil	8 years	nil	3 years	nil
Numbers of separate lesions examined	7	3	2	6	6	2
histologically in present study						
Distribution of lesions	Cheeks; back and sides of neck	Face; back of neck	Head and neck only	Face and neck	Face, neck and upper chest; scars forearm extensor surfaces	Face, neck; few on extremities
Lesions removed by others	4	nil	Several	Several	Several	Few
Features and associations	Tumours preceded by hard lesion at hair root. Sclerotic scars with marginal warty relapses	Associated eczema. White scars Milia	Onset with 'follicular plug'. Nodules to warty patches. Sclerotic scars. Erysipelas once as complication	Night blindness	Scars suggest excoriations. Onset with abating cuts. Mental hospital inpatient	Much sun exposure
Diagnostic suggestions from elsewhere	Inverted follicular keratosis; prurigo nodularis; acne; trichoepithelioma	—	'Benign'	Inverted keratosis. Prurigo	—	Keratosis
Treatments attempted by various colleagues	Excision, curettage intralesional triamcinolone	Steroids with occlusion	Curettage	Freezing; on etretinate lesions flatten, discharge, and involute	Excision	Freezing; curettage

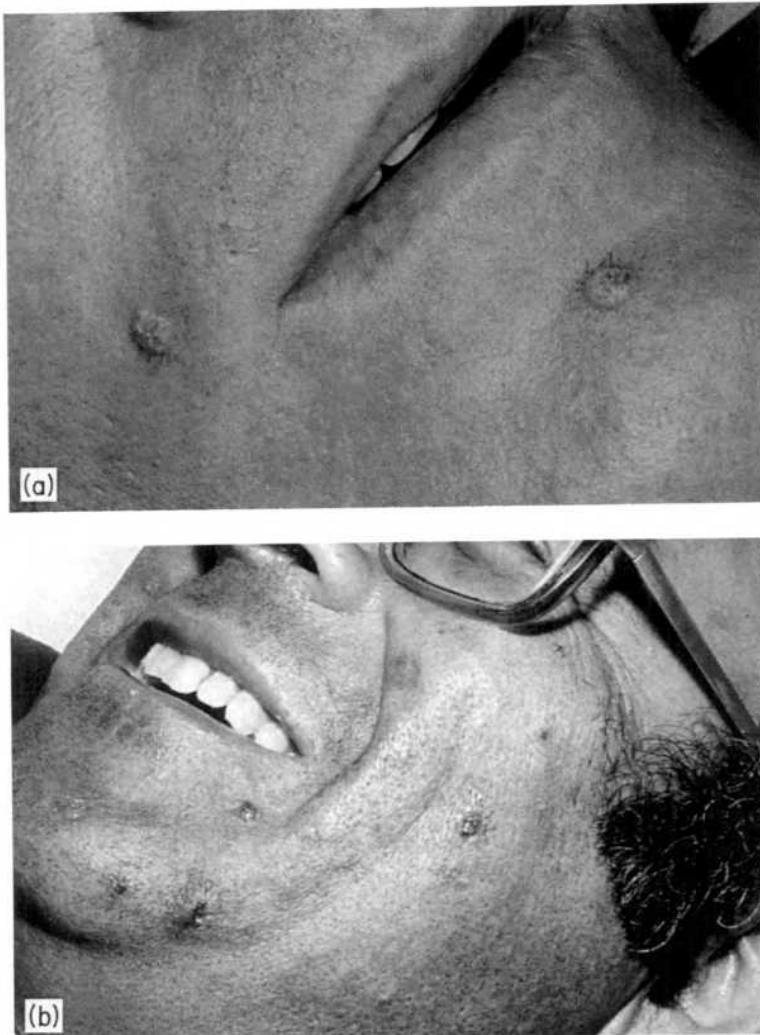


FIGURE 1. (a) Case 2: two undamaged lesions on chin and nasolabial fold. (b) Case 4: active crusted and scarred lesions over the cheeks and chin.

HISTOLOGY

Twenty-five separate lesions were studied histologically on routine paraffin-embedded haematoxylin-and-eosin-stained sections (Fig. 2 a-c). Several specimens were examined from each patient.

General pattern

The distinctive pattern in the growths was a cluster or bunch of recesses in the epidermal surface. These inwardly projecting sacs were U- or V-shaped, polypoid, bilobed, or bifurcate. They were linked by short bridges or epidermis at the surface. The structure and position of these recesses corresponded most closely to that of an enlarged and widened follicular infundibulum. A central hair was lacking, although an occasional vellus hair was noted which

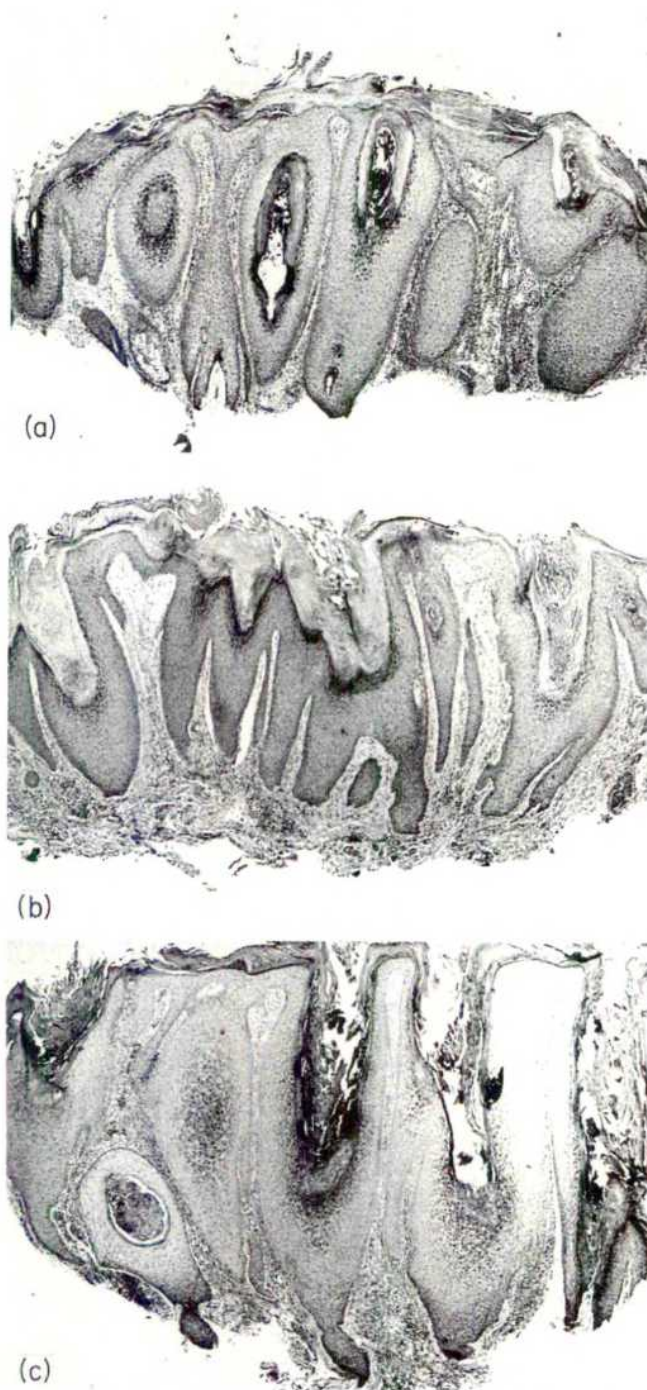


FIGURE 2. (a) Three infundibular components of the tumour. A normal hair follicle penetrates from below. Surface crusting and sebaceous gland tissue shown (Case 2). (b) Infundibula showing broad and bilobed structures, and hypergranulosis (Case 4). (c) Lesion from forehead with intact surface. Closely adjacent infundibula; dilated papillary vessels between (Case 5). (Original $\times 40$.)

was disproportionately small for the size of the infundibulum. The tumour could radiate so as to give either an exophytic appearance, or an endophytic type of growth. The tumour components ended below at about the top of the reticular dermis, at a level where elastosis commences. A few sebaceous-type cells were often generated at this point. Occasionally these sacs were more deeply connected with other follicular structures, but these deeper parts were usually missing or separate from the growth.

The units

The saccular elements of the tumour comprised an epithelial basal layer, with some suprabasal proliferation in places. In some sites there were many mitoses but with no hint of atypicality. Whorling or eddy-formation among the epithelial cells was not seen. The Malpighian layer was non-vacuolated and thick and a very considerable and prominent granular layer extended well down the infundibulum. A laminated or wedged, dense orthokeratotic keratin plug filled the central space.

Epithelial cords

A minor feature was the presence of narrow epithelial cords without much suprabasal differentiation. These cords descended from the epidermis, ending blindly or anastomosing with some of the larger infundibula, or sprouted from the sides of the infundibula, pointing downward to the depths.

Stroma

The loose stromal connective tissue showed slight fibre production, some widely dilated vascular channels, and a pale-staining oedematous structure with slight lymphohistiocytic increase. It penetrated between the infundibula and the epithelial cords forming extremely long and slender strands. These same processes also penetrated the infundibula in places, rather like the papilla of a hair follicle. In this way a fenestrated appearance was sometimes produced.

Inflammatory changes

Signs of impetiginization, trauma and slight eczematization were seen on the narrow surface strips of epithelium—neutrophils, blood, oedema, crusting, patchy parakeratosis, erosions, etc. In the stroma of a few specimens, plasma cell accumulations and the occasional giant-cell granuloma were seen.

Late changes

In the possibly older and more burnt-out lesions were milia, thin-walled keratinous retention cysts containing loose keratin and formed in the lower parts of the abnormal infundibulum. In these older lesions there was fine wavy collagen in the tumour stroma or horizontally arranged layers of scarring. Surface discharge occurred by exophytic eversion of the infundibulum and a persistent intraepidermal infundibulum, the strictly follicular poroma, showed itself as an acanthotic epidermis with a granular layer up to 15 cells thick.

In certain specimens, the picture of tapering infundibular recesses was also partly replaced by block-like acanthomatous developments with little cavitation and a thick granular layer. In one specimen an irregularly indented hypergranular surface layer was associated with deep-seated milia in the infundibula.

DISCUSSION

It was difficult to regard these lesions as variants of ordinary warts, prurigo nodules or neurodermatitis as well as any of the solitary or genetically induced tumours. They resembled the sporadic form of Ferguson Smith self-healing epithelioma. Their origin was superficial and appeared to be derived from a cluster of newly-arising structures that resembled the follicular infundibulum.

The benign follicular tumours have been the object of extensive review¹ and of 'controversy'.² Their family relationships, stressing the grades, materials, and degrees of differentiation between the tumour types, are scarcely established. The condition described in this paper resembles closest that of follicular poroma,³ or else to a tumour of the follicular infundibulum.⁴ However, the former corresponds only to the intra-epidermal 'hair canal' portion, and the latter to a subepidermal plate of abnormal tissue. Though Mehregan and Butler⁵ enjoy priority for describing infundibular tumours under this particular title, the infundibulum was only partially simulated in their material. Our cases should be regarded as representing the entire infundibular unit, as conceived by Pinkus.⁶ The biological validity and independence of this trumpet-like segment of the follicle is thus supported. At this stage the derivation of these lesions from a papilloma is uncertain. The papillary processes seen in these tumours are the least involved in any proliferation.

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Isotretinoin differs from other synthetic retinoids in its modulation of human cellular retinoic acid binding protein (CRABP)

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SUMMARY

Isotretinoin differs from acitretin and Ro137410 by its striking sebostatic effect in acne after oral, but not topical, administration. The reason for this is not yet understood.

Previous studies indicate that cellular retinoic acid binding protein (CRABP) might be implicated in the action of synthetic retinoids. We, therefore, compared the three retinoids for their ability to increase epidermal CRABP levels after systemic and topical treatment. Oral treatment with acitretin and Ro137410 led to a striking increase of epidermal CRABP (from 2.6 ± 0.9 to 16.2 ± 2.9 , $P < 0.004$ and from 2.5 ± 1.2 to 21.5 ± 3.4 pmol/mg protein, $P < 0.004$, respectively), while isotretinoin failed to induce a comparable rise (3.2 ± 1.6 before and 3.7 ± 0.7 pmol/mg protein after treatment), although it displayed in all patients a striking sebostatic effect. After topical application, the increase of CRABP was comparable for all three compounds. The interaction of isotretinoin with the epidermis seems to be different from other synthetic retinoids only after systemic treatment, a finding that parallels clinical observations.

In the last few years reports about the benefit of isotretinoin in the treatment of acne vulgaris have been accumulating.^{1–2} The overall experience with the systemic use of this drug has been good, and its efficacy attributed partly to its anti-inflammatory effect,³ but mainly to its striking sebostatic action.^{4–5} Isotretinoin, however, when applied topically, loses its sebostatic property,⁶ though there remains some anti-inflammatory effect.⁷

So far no other retinoid has been found with a similar effect on sebum secretion. Neither tretinate, acitretin nor Ro137410 have shown any sebostatic effect⁸ and up till now it is not understood why there should be this striking difference in action between substances that are structurally so similar.

Cellular retinoic acid binding protein (CRABP) is a protein that transports specifically

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retinoic acid from cytosol to nucleus.⁹ Apart from the reproductive organs it is most abundant in the skin.¹⁰⁻¹¹ Its role and regulation is not yet quite understood.¹² Elevated levels of epidermal CRABP have been found in cutaneous disease¹³ and after systemic retinoid treatment.¹⁴

Assuming that CRABP might be implicated in the action of synthetic retinoids, we compared isotretinoin with acitretin and Ro137410 for their ability to increase epidermal CRABP levels. Acitretin, the free acid of etretinate, exerts rather similar effects and side effects to etretinate and has been used in the treatment of psoriasis and other dyskeratoses.¹⁵ Ro137410, an arotinoid acid, is the main metabolite of arotinoid ethyl ester, Ro136298, and proved to have antihyperkeratotic properties.¹⁶ We investigated the effects of these compounds on epidermal CRABP after systemic and topical treatment. We found that isotretinoin differed from other retinoids in a unique way when given orally but not after topical administration.

METHODS

Systemic treatment

A total of nine acne patients (seven males and two females) with an average age of 20 years (15 to 27 years) were treated orally with isotretinoin (0.5 mg/kg a day). Female patients received concomitant oral contraception during the treatment. After informed consent an epidermal sample was taken before treatment, in five patients, after 1 month and two months and in four patients after 2 months of treatment. The epidermal samples were taken with a keratome from a non-lesional site on the buttock under local anaesthesia as previously described.²³

Four patients (two females, two males) with an average age of 58 years (28-71 years) were treated with 0.6 ± 0.05 µg/kg a day of Ro137410. One patient was treated for lamellar ichthyosis, one for nevroid basal cell carcinoma syndrome and two for generalized prurigo. After informed consent an epidermal sample was taken from non-lesional skin, before and 4 weeks after start of treatment. One of the patients with prurigo was treated twice in a 5-month interval.

Five patients with psoriasis and one with lichen planus (five males, one female; mean age 38 years, ranging from 20 to 48 years) were treated with acitretin in a dose of 0.65 ± 0.04 mg/kg a day. Epidermal samples were obtained before and 4 weeks after start of the treatment from non-lesional skin.

Repeated topical application

One hundred microlitres of the test solution and its solvent were applied on the right and left buttock of healthy human volunteers, covering a square approximately 2×2 cm each. The sites were put under occlusion and the same procedure was repeated during 4 days. On the fifth day epidermal specimens were taken from both sides with a keratome using local anaesthesia (with 1% xylocaine). The samples were immediately frozen in liquid nitrogen.

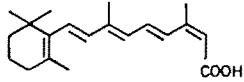
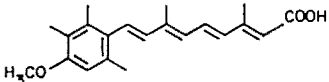
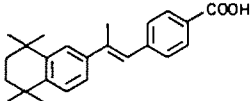
Substances

Table 1 shows the test substances and their solvents that were locally applied at a concentration of 0.1% corresponding to 0.3 µmol/ site. The test doses had been arbitrarily chosen taking into consideration the clinically applied topical concentration of tretinoin.

Preparation of supernatants

About 15 mg of lyophilized skin samples were full-speed homogenized during 3×30 s in 400 µl extraction buffer (TRIS 50 mM, NaCl 25 mM, EDTA 2.5 mM, pH 7.5) using a Polytron-PT7 homogenizer (Kinetica, Lucerne, Switzerland). Supernatants were obtained by centrifugation

TABLE 1. Topically applied substances and their solvents

Ro-No	Name	Basic chemical structure	Solvent
4-3780	isotretinoin		ethanol
10-1670	acitretin		ethanol + 10% DMSO
13-7410	arotinoid, free acid		ethanol + 10% DMSO

at 100,000 *g* for 60 min at 4°C. The total protein content of the supernatants was determined by a colorimetric method.¹⁷

Determination of CRABP

After overnight incubation of the supernatants with 600 nM of tritiated all-trans retinoic acid (51.6 Ci/mmol, MEM Dreieich, FRG), the binding proteins were quantified by a polyacrylamid gel electrophoresis (PAGE) as previously described.¹⁸ After migration the gel was cut into 2 mm-slices and the radioactivity of each slice assessed in a liquid scintillation counter (Beckmann LS-1800) using 4 ml Biofluor (New England Nuclear). Binding activity was expressed as pmol of the radioactive ligand specifically bound per mg of total protein or DNA content.

Statistics

Results are expressed as the means $\pm$ standard error of the mean. Statistical significance was calculated by the paired Student's *t*-test.

RESULTS

Systemic treatment

Acitretin and Ro137410 dramatically increased epidermal CRABP after 4 weeks of oral treatment (Fig. 1). CRABP rose from 2.6 ± 0.9 to 16.2 ± 2.9 pmol/mg protein ($P < 0.004$) after acitretin treatment and from 2.5 ± 1.2 to 21.5 ± 3.4 pmol/mg protein ($P < 0.004$) after Ro137410. Isotretinoin did not show a comparable effect (Fig. 1). CRABP remained unchanged after 1 month of treatment (3.2 ± 1.6 before and 3.7 ± 0.7 pmol/mg protein after treatment) and increased insignificantly after 2 months (4.4 ± 0.5 pmol/mg protein). In all patients treated with isotretinoin a striking sebostatic effect was observed. Total protein per mg tissue was not significantly different before and after treatment.

Topical application

After 4 days of repeated application of acitretin, isotretinoin or Ro137410, there were no signs of irritation of the skin which appeared normal. On histology there was no difference between the treated and control areas. CRABP levels, however, were significantly increased in the retinoid treated samples (Fig. 2). After topical application the isotretinoin effect on epidermal CRABP was comparable to that of acitretin or Ro137410 contrasting with the results obtained after systemic administration. Total protein content per mg tissue was comparable in treated and solvent-treated samples.

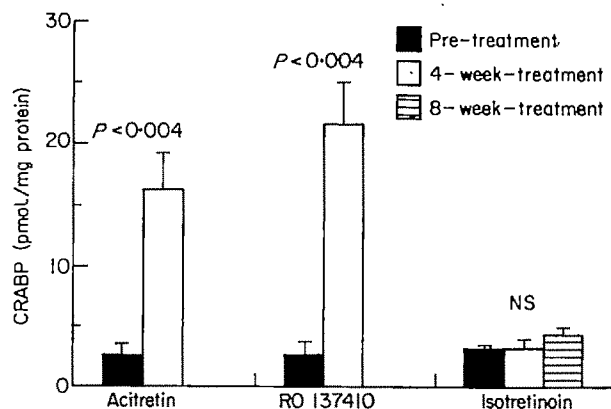


FIGURE 1. Epidermal CRABP after systemic treatment. Values are expressed as mean $\pm$ SEM. Statistical significance according to the paired Student's *t*-test is represented in numbers above columns.

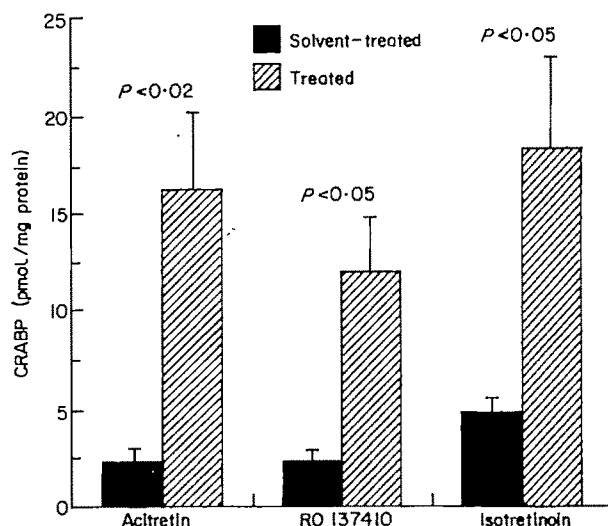


FIGURE 2. A 0.1%-solution of each drug or its solvent were applied topically once a day during 4 days before epidermal CRABP was determined in the drug- and solvent-treated area. Values are expressed as mean $\pm$ SEM. Statistical significance according to the paired Student's *t*-test is represented in numbers above columns.

DISCUSSION

In the present study we observed an increase of the epidermal retinoic acid binding protein after systemic treatment with the retinoid RO137410 and acitretin. This confirms our previous observations.¹⁴

Topical application of two of these retinoids for 4 days increased epidermal CRABP to the same extent. Previous experiments¹⁹ have shown that acitretin increases epidermal CRABP within 24 h after one single topical application, and after repeated applications over a period of 4 days, a steady state is reached. The comparable increase of epidermal CRABP after systemic

and topical application of both drugs suggests that the two drugs have a direct effect on epidermal CRABP and if metabolized, this also takes place in the epidermis. This does not apply for isotretinoin. Although it increased CRABP after topical application, it did not do so after systemic treatment. To exclude a delayed effect we determined epidermal CRABP not only after 4, but also after 8 weeks of treatment, but there was no significant difference between the pre- and post-treatment levels.

Why does isotretinoin not act like the other retinoids on epidermal CRABP when given orally? Firstly, isotretinoin might not reach the epidermis in sufficient quantities to stimulate a rise of CRABP. Vahlquist has measured a drug level of 69 ng/g wet tissue in the epidermis after 1 month of oral isotretinoin treatment²⁰ and between 100 and 200 ng/g wet weight after 1 month of etretinate treatment including its main metabolite acitretin.²¹ This could mean that a great amount of isotretinoin does not reach in the epidermis. Or is it metabolized to a substance that is not able to interact with CRABP?

It is possible that isotretinoin does not interact with CRABP at all and that the increase of CRABP after topical application is due to isomerization of 13-cis into all-trans retinoic acid (RA). In fact, Lehman has shown²² that 30% of topically applied 13-cis RA is photodegraded into all-trans RA and that this ratio is consistent throughout the skin, suggesting that no further isomerization takes place in the skin itself. We have shown in previous experiments that topical RA increases epidermal CRABP.¹⁹ Thus, the observed increase could be due to the presence of RA that is inevitably produced with topical application. When given systemically and with no exposure to light endogenous isomerization is low²³ and neither dermis nor epidermis seem to be able to actively isomerize the drug.²²

Why isotretinoin does not interact with CRABP in contrast to acitretin and Ro137410, is not clear. The affinity of isotretinoin to rat CRABP is 30 to 50 times lower²⁴ than that of RA and affinity tests with short incubation times as carried out in our laboratory with human epidermal CRABP suggest that there is no binding.²⁵ We have shown²⁶ that Ro150778, a retinoid without any binding capacity to CRABP, does not affect epidermal CRABP. Affinity to CRABP is probably not the only prerequisite a retinoid must have to increase epidermal CRABP levels. Our previous experiments suggest that induction of biological responses or recruitment of high amounts of RA might also influence CRABP levels.¹⁹

Acitretin and Ro137410 on the one hand, and isotretinoin on the other hand are characterized by distinct clinical and therapeutic properties which overlap only when isotretinoin is given in high doses (2 mg/kg a day). Isotretinoin has a marked sebostatic effect when given even in small doses (0.1 mg/kg a day) which is not seen with the other drugs.

An analysis of the side-effects of isotretinoin and etretinate²⁷ shows that with etretinate the cutaneous side effects that do not concern the face are more important. Peeling of palms and soles, areas without sebaceous glands, is marked after etretinate, but not seen with isotretinoin. The therapeutic effect on hyperkeratotic diseases with isotretinoin can only be achieved with high doses (2 mg/kg a day).^{28,29} These doses may increase its epidermal effects and it cannot be excluded that epidermal CRABP might be altered as well.

The biological significance of the retinoid-induced increase of epidermal CRABP is not yet understood. The hypothesis that CRABP increase might occur in situations of exogenous or endogenous retinoic acid overload is discussed elsewhere.¹⁹ CRABP modulation could be interpreted as an indicator of retinoid action on the epidermis. Our results seem to confirm the clinical observations that isotretinoin has poor biological affinity for the epidermis, but is a very specific inhibitor of sebaceous gland activity.

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An investigation into the incidence of auto-immune disorders in patients with localized morphoea

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SUMMARY

Patients with localized morphoea have an increased incidence of auto-immune disorders, but this is not related to the age of onset, duration of disease nor the site or number of lesions. The patients have an increased incidence of tissue antibodies and these are more common when more than one lesion is present. The relatives of the patients have also been found to have an increased incidence of auto-immune disorders.

These findings suggest an immunological basis for the disease and indicate that the patients should be investigated and followed up to detect the evidence of other auto-immune disorders.

Morphoea is a disorder of unknown aetiology in which localized sclerosis of the skin occurs. Linear and plaque lesions occur and the ratio of affected females to males is 3:1. Seventy-five per cent of the linear lesions arise before the age of 40 years while 75% of plaque lesions occur between the ages of 20 and 50 years.¹ There are clinical and histological similarities between morphoea and lichen sclerosus et atrophicus (LSA) and the two conditions may coexist.²

A study on patients with LSA has shown that they suffer from an increased incidence of other auto-immune disorders³ and this prompted a similar study in patients with localized morphoea. The results are compared with those found in the LSA group.

METHODS

Over a period of 5 years, 50 Caucasian females with histologically proven morphoea have been investigated. The patients were compared with 50 individually age matched controls who were attending the skin clinic for minor complaints and were investigated concurrently. All the patients and controls were asked if they suffered from pernicious anaemia, hypothyroidism, thyrotoxicosis, alopecia areata, diabetes mellitus or vitiligo. An enquiry was also made into whether any first, second or third degree relative suffered from any of these conditions. If necessary, hospital records were obtained to verify the diagnoses.

TABLE 1. Details of 50 patients with morphea

Age at presentation	4-77 years	(Mean 36.8)
Age at onset of symptoms	4-67 years	(Mean 34.7)
Duration of symptoms at presentation	5 weeks-19 years	(Mean 31.9 months) (Median 9 months)

The investigations performed were anti-nuclear factor, smooth muscle antibody, anti-mitochondrial antibody, thyroid antibodies, gastric parietal cell and intrinsic factor antibodies, fasting blood sugar and HLA type. When positive antibody results were obtained, investigations were performed to exclude the relevant disease.

The age at presentation of the patients ranged from 4 to 77 years (mean 36.8, SD 18.4, SEM 2.6) and are shown in Table 1. The age at onset of symptoms was 4 to 67 years (mean 34.7, SD 18.7, SEM 2.6) and the duration of symptoms at presentation varied enormously from 5 weeks to 19 years (median 9 months, mean 31.9 months, SD 60.13, SEM 8.5).

The age of onset was divided into three groups, those who were: (1) under 20 years (16 patients); (2) 20 to 39 years (11 patients); (3) over the age of 40 (23 patients). Twenty-seven patients had lesions of morphea for less than 1 year at presentation and 23 had lesions for more than a year.

Of the 50 patients studied, 47 had plaque lesions and three had linear limb lesions. Lesions were on the trunk in 34 patients, on limbs only in eight patients and both trunk and limbs were involved in eight patients. Thirty-three patients had a single lesion and 17 had more than one lesion.

RESULTS

Eight patients (16%), were suffering or had suffered from an auto-immune disease as compared with one of the controls. Fisher's exact test⁴ shows that there is a significant difference ($P < 0.02$) (Table 2). However, in this small group, when the conditions are analysed individually, none of the disorders are significantly more common in the patient group.

TABLE 2. Presence of other disorders

	Patients (50)	Controls (50)	P value
With at least 1 disorder	8	1	<0.02
Individual disorders			
Pernicious anaemia	2	0	NS
Hypothyroidism	1	0	NS
Thyrotoxicosis	1	0	NS
Alopecia areata	1	0	NS
Diabetes	2	1	NS
Vitiligo	1	0	NS

NS = Not significant.

TABLE 3. Family history

	Patients (50)	Controls (50)	P value
With at least one relative suffering from an auto-immune disease	20	5	$P < 0.001$
Individual disorders			
Pernicious anaemia	3	2	NS
Hypothyroidism	5	0	$P < 0.03$
Thyrotoxicosis	5	1	NS
Alopecia areata	4	0	$P < 0.06$
Diabetes	11	2	$P < 0.01$
Vitiligo	2	0	NS

NS = Not significant.

One of the cases of pernicious anaemia was diagnosed as a result of this study, and interestingly, two patients also had rheumatoid arthritis and one patient was found to have Addison's disease as a result of the investigation. Both of these disorders may be considered as being auto-immune in origin.

Statistical analysis using the Chi-square test showed that the incidence of auto-immune diseases in patients with morphoea was not related to the age of onset, the duration of disease, or the site or number of lesions.

Enquiry into the family histories revealed that 20 patients had relatives suffering from a total of 30 auto-immune disorders (Table 3). Only five controls had relatives affected. A Chi-square test revealed that this overall difference was significant ($P < 0.001$). When the conditions were analysed individually by use of the Fisher test, it was seen that hypothyroidism, alopecia areata and diabetes are significantly more common in relatives of patients with morphoea than in relatives of controls. The age of onset, duration of symptoms and site and number of lesions bore no significant relationship to the incidence of auto-immune disease in the families of patients with morphoea.

TABLE 4. Tissue antibodies

	Patients (50)	Controls (50)	P value
With at least one tissue antibody	26	4	$P < 0.0005$
Individual disorders			
Antinuclear factor	22	3	$P < 0.0005$
Smooth muscle antibody	3	0	NS
Thyroid antibodies	7	1	$P < 0.03$
Gastric parietal cell antibody	5	0	$P < 0.03$
Intrinsic factor antibody	2	0	NS
Positive antibodies were significantly more common in patients with more than one lesion ($P < 0.025$).			

NS = Not significant.

TABLE 5. Comparison of morphoea and LSA results

	Morphoea (Total 50)	LSA (Total 50)	P value
At least one disorder	8	17	$P < 0.04$
At least one relative suffering from an A-1 disorder	20	18	NS
With at least one tissue antibody	26	37	$P < 0.03$

NS = Not significant.

Twenty-six patients had at least one tissue antibody compared with four controls, a highly significant difference ($P < 0.0005$, Table 4) from a Chi-square test. Nine patients had more than one positive antibody and there was a total of 39 positive results in the patient group. Taken individually, anti-nuclear factor, thyroid antibodies and gastric parietal cell antibodies were significantly more common in patients with morphoea. The presence of tissue antibodies was not related to the age of the patient, or the duration or site of the lesion, but positive antibodies were significantly more common in patients with more than one lesion ($P < 0.025$).

For the investigation of HLA types, the group of 50 patients was compared with a Sheffield population sample of 3000. There was no antigen found to be significantly more common in the patients with morphoea. This differs from our study of the LSA patients where HLA B40 was found to be significantly more common in that group.

When comparing the other results with those found in the LSA group (Table 5) Chi-square tests revealed that the incidence of other auto-immune disorders and positive tissue antibodies is significantly higher in the LSA group, but that there is no significant difference in the incidence of affected relatives.

DISCUSSION

This study is the first to show that patients with localized morphoea have an increased incidence of auto-immune disorders, but this is not related to the age of onset or duration of disease, nor to the site or number of lesions. These patients also have an increased incidence of tissue antibodies and these are more common in patients with more than one lesion. The patients' relatives have also been found to have an increased incidence of auto-immune disorders.

These findings, together with the fact that morphoea-like lesions are seen in graft versus host reactions, suggest an immunological basis for morphoea. The results are similar to those found in the LSA patients, but there is not a common HLA association for the two disorders.

Patients with localized morphoea should be investigated and followed up to detect the existence of other auto-immune diseases.

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Acne is not associated with abnormal plasma androgens

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SUMMARY

Plasma dehydroepiandrosterone sulphate, androstenedione, testosterone (T), dihydrotestosterone (DHT), and sex hormone binding globulin (SHBG) have been measured in 64 females and 26 males aged less than 25 years and with acne vulgaris. Oestradiol was measured in the males. Free T and free DHT were calculated. Acne was graded on three sites and the sebum excretion rate (SER) was measured in most patients. With the possible exception of free DHT, none of the plasma steroids or SHBG correlated with acne severity or with SER. Free DHT in the females showed a possible, but weak, correlation with total acne ($r=0.25$, $P=0.07$), but comparison with male data showed that this was not causative. The role of androgens in acne is permissive and plasma androgen measurements usually have no place in its management.

There is strong but mostly circumstantial evidence that the development of acne vulgaris depends upon the presence of critical levels of androgens. Pre-pubertal children do not suffer from acne, but administration of androgens to children produces growth of the sebaceous glands¹ on which acne depends.^{2–4}

It has often been suggested that androgens have more than a simple permissive role in the development of acne. It has been argued that severe clinical acne is often associated with abnormally high androgen levels. Thus, several studies of female acne patients have shown elevations of plasma androgens compared with controls. However, the prevalence of either physiological or clinical acne is 90% or more in both male and female teenagers and is still over 50% at 30 years of age.⁵ This high prevalence calls into question the status of the 'control' subjects in these studies.

A less contentious approach is to relate androgen concentrations to the grade of acne within groups of patients. If high androgen levels are important as a causative factor in severe acne, there should be correlations between androgens and acne grade.

Darley *et al.*⁶ found that neither sex hormone binding globulin (SHBG) nor dehydroepiandrosterone sulphate correlated with acne severity. In contrast, Odland *et al.*⁷ and Lawrence *et*

*al.*⁸ found lower levels of SHBG (implying higher levels of free testosterone) in severe acne as compared with mild cases. This study has been carried out on patients with a wide range of severity of acne ranging from mild to moderate and severe.

METHODS

All the subjects had been referred with acne as their only complaint. There were 64 females aged 12–24 years (mean age 19.3) whose acne began before the age of 19 (mean age of onset 13.1 years) and 26 males, aged 15–26 years (mean age 20.6) whose acne began before the age of 17 (mean 13.9 years). All subjects had been off treatment for 4 weeks prior to the investigation. The acne severity was assessed under good lighting by the technique of Burke and Cunliffe.⁹ The face, back, and chest grades were totalled to give a total acne score.

Any facial hirsutism was noted. In 66% of the female patients, a detailed menstrual history was taken and in 45% of them their hair growth was graded.¹⁰

The female patients were divided into 'mild' and 'moderate to severe'. This was to provide an alternative to regression analysis for examining the relationship of androgen levels to acne grade. 'Mild' patients showed scores of less than 1.5 at each site and a total of the three sites of less than 2.0.

SER was measured in 44 of the females and 12 of the males as previously described.¹¹

Blood was taken at 09.00 h and dehydroepiandrosterone sulphate, androstenedione, testosterone, and dihydrotestosterone (DHT) were measured by established in-house radio-immunoassay methods in the Leeds supraregional assay laboratory, monitored by both internal

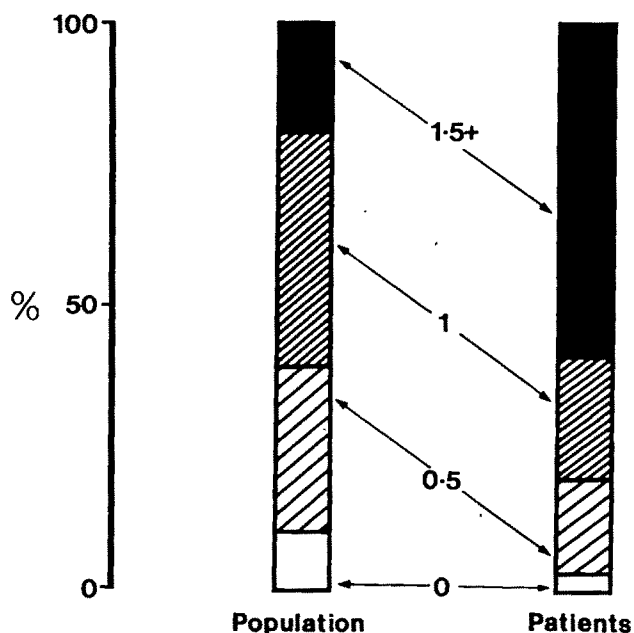


FIGURE 1. Distribution of facial acne scores in the female patients and in the female population at age 19. The population data are from Cunliffe and Gould⁵ and are recalculated using the current acne grading scale.⁹

and U.K. national quality assurance schemes. Sex hormone binding globulin (SHBG) was measured by radial immunodiffusion.¹² Free testosterone was calculated from testosterone and SHBG;¹³ free DHT was calculated using the same formula but substituting association constants for DHT.¹⁴

Statistical analysis was performed using the MINITAB computer package and means were compared by Student's *t*-test. Rank correlations were used when the distributions appeared non-Gaussian and for acne scores.

RESULTS

Female

The distribution of facial acne scores is shown in Figure 1, where it is compared with the distribution of scores found by Cunliffe and Gould,⁵ when they examined an unselected group of healthy subjects drawn from the same community as the patients. While the acne scores of back and chest correlated with each other ($r = 0.58$, $P < 0.01$), both showed poor correlation with facial acne ($r = 0.15$ in both cases). Only one patient had significant hirsutism (a Ferriman and Gallwey¹⁰ score of 15), and of the 29 patients on whom formal hirsutism scores were made, four rather than the two expected from the data of Ferriman and Gallwey had scores above 7.

TABLE 1. Summary of data as means $\pm$ SD

	All	Females	Males	
		Mild acne	Moderate/severe acne	
<i>n</i>	64	20	44	26
Age (years)	19.3 $\pm$ 2.9	18.9 $\pm$ 3.8	19.4 $\pm$ 2.6	20.6 $\pm$ 3.0
Duration (years)	6.1 $\pm$ 3.3	6.9 $\pm$ 4.2	5.9 $\pm$ 3.0	7.0 $\pm$ 3.7
Acne score—Face	1.9 $\pm$ 1.4	0.7 $\pm$ 0.4	2.3 $\pm$ 1.6*	2.0 $\pm$ 1.4
—Total	3.2 $\pm$ 2.1	1.2 $\pm$ 0.5	3.8 $\pm$ 2.0*	6.0 $\pm$ 3.9†
SER ($\mu\text{g}/\text{cm}^2\text{min}^{-2}$)	1.2 $\pm$ 0.4	1.3 $\pm$ 0.3	1.2 $\pm$ 0.5	1.8 $\pm$ 0.4
	(44)	(11)	(33)	(12)
DHAS ($\mu\text{mol}/\text{l}$)	6.4 $\pm$ 2.8	7.9 $\pm$ 4.4	6.0 $\pm$ 1.9	7.9 $\pm$ 3.4
Androstenedione (nmol/l)	7.8 $\pm$ 2.4	8.8 $\pm$ 2.1	7.4 $\pm$ 2.4	9.5 $\pm$ 3.1†
	(31)	(9)	(22)	
Testosterone (nmol/l)	1.6 $\pm$ 0.6	1.8 $\pm$ 0.8	1.5 $\pm$ 0.5	22.8 $\pm$ 7.3†
DHT (nmol/l)	0.74 $\pm$ 0.35	0.68 $\pm$ 0.45	0.76 $\pm$ 0.32	2.6 $\pm$ 1.0†
	(54)	(10)		
SHBG (nmol/l)	56 $\pm$ 32	60 $\pm$ 53	55 $\pm$ 23	34 $\pm$ 10†
Free T (pmol/l)	26 $\pm$ 13	31 $\pm$ 18	24 $\pm$ 11	523 $\pm$ 173†
Free DHT (pmol/l)	2.6 $\pm$ 1.5	2.5 $\pm$ 1.6	2.6 $\pm$ 1.5	14 $\pm$ 7†
	(54)	(10)		
Oestradiol (pmol/l)	—	—	—	121 $\pm$ 3.8

DHAS = dehydroepiandrosterone sulphate, free T = free testosterone.

'Mild' patients had grades of < 1.5 (scale of Burke and Cunliffe⁹) at each site.

'Moderate/severe' patients had a grade of 1.5 or more on at least one site.

Results of tests of significance * $P < 0.01$ 'moderate/severe' compared with 'mild'

† $P < 0.01$ males compared with females.

Numbers in parentheses = *n* where this differs from the total number of patients.

One-third of the patients questioned had irregular periods. These had a lower mean SHBG (43 nmol/l, compared with 62 nmol/l) and a higher mean free testosterone (32 pmol/l, compared with 22 pmol/l) when compared with the patients who had regular periods.

One-third of the patients were on some form of oral contraceptive (though none was on a triphasic or anti-androgen containing preparation). As a group, they had raised SHBG (74 nmol/l compared with 49 nmol/l in those not on oral contraceptives) and lower androstenedione (6.2 nmol/l compared with 8.6 nmol/l), testosterone (1.2 nmol/l compared with 1.7 nmol/l) and free testosterone (16 pmol/l compared with 29 pmol/l). The acne grades did not differ significantly in those who were on oral contraceptives and those who were not taking them and in those with regular or irregular menstruation.

With the possible exception of free DHT, none of the androgens, or SHBG, correlated with acne scores at any site or with total acne score or with SER. Free DHT showed a possible weak correlation with total acne in the females ($r=0.25$, $P=0.07$). There were no significant differences of any androgen or of SHBG between the 'mild' and 'moderate-severe' subgroups (Table 1).

Male

As with the females, none of the androgens or the oestradiol or the SHBG correlated with any acne scores or with the SER. However, the SER correlated with the facial acne score ($r=0.76$, $P<0.01$).

DISCUSSION

Most young people have acne. In that sense, it is normal. If there is an abnormality it is perhaps the total absence of acne.³

The unusual features of the present series compared with most published studies are the wide range of acne grades and the low prevalence of hirsutism among the patients. These features result from one of us (WJC) being well-known to family doctors for his interest in acne and his low threshold for accepting acne patients. Moreover, in the U.K., there is at present no financial disincentive to seeking medical help.

Figure 1 compares the facial acne scores of the 64 female patients with data from Cunliffe and Gould⁵ for the population from which the patients were drawn. These data used a different grading system but have been replotted using the current system.⁹ Many of the patients, although complaining of acne, had relatively mild grades comparable with the acne of a high proportion of women of this age group. This allowed us to select from among the patients a reference group with mild acne against which the patients with moderate to severe acne may be compared, the two groups being otherwise similar in all respects.

The present data show little evidence of high androgens causing clinical acne. With the possible exception of DHT, none of the androgens correlated with acne severity and none differed significantly between the 'mild' and 'moderate to severe' subgroups. Darley *et al.*⁶ also reported no correlations between acne severity and any of dehydroepiandrosterone sulphate, testosterone, or SHBG. In contrast, Odland *et al.*⁷ and Lawrence *et al.*⁸ reported lower SHBG concentrations in severe, compared with mild, acne. However, like ourselves, Lawrence *et al.*⁸ found no difference of free testosterone between mild and severe subgroups.

Most reported claims that androgens are 'abnormal' in patients with acne are based upon the comparison of patient groups and normal control groups. Increases of testosterone have been reported^{6,15-20} but others have reported normal testosterone.^{7,8,21,22} A decrease of SHBG (with an increase of free testosterone either measured or implied) has been reported.^{6,7,16,18,22} Small

increases of dehydroepiandrosterone sulphate have been found.^{6,15,17-22} DHT was not increased,^{8,22} but its metabolite, androstenediol, was increased.²³

We suggest that there is a fundamental methodological flaw in the comparison of patient and normal control groups. The problem stems from the high prevalence of acne in the general population. This has two consequences. Firstly, 90% of 20-year olds in a control group are likely to have acne. If they have been selected by a dermatologist as having no acne, they are no longer 'normal controls'; instead they are the unusual 10% of adolescents who have no acne. Secondly, not all subjects with acne reach a dermatology clinic. Those that do are, therefore, selected. Most reported acne series show a high prevalence of hirsutism (e.g. up to 50%) suggesting that co-existence of an androgen-dependent disease is one factor leading to selection. It is not surprising that in such series of patients that they have high androgens. Among our own female patients a large proportion had irregular periods and they showed higher testosterone values. It is true that in several reports values from women with hirsutism are listed separately, but unless we can identify all the factors contributing to selection, it is unsafe to assume that none of them has affected the androgen levels.

Another potential source of differences between patients and controls is that the circumstances of sample collection or analysis may differ between them. Clearly, in a well-organized study, one would expect such differences to be minimal. However, it is difficult to exclude the possibility of stress affecting patients more than controls. Moreover, studies in which controls and patients were measured at different times may have been affected by small differences due to changes of analytical method bias. Radioimmunoassay is notoriously difficult to control in this respect.

Our data would be consistent with a weak relationship between acne and free DHT. However, it is important that this correlation should not be regarded as implying causation. Male DHT levels are much higher than female levels. Even the males with the lowest acne grades had mean DHT levels of at least three times that of the females with severe acne. Clearly, circulating DHT is not causing acne. However, DHT arises partly by peripheral conversion from testosterone and the results are consistent with greater rates of conversion in skin containing active sebaceous glands.

A well-known feature of acne, which is illustrated by our measurements, is the poor correlation between acne grade at different sites. It is surprising that the inference from this—that acne is due to local rather than systemic factors—has been so little recognized.

The male patients showed the expected correlation² between acne grade and SER. The failure of our female patients to show a significant correlation may have been due to the smaller numbers and to having subjects only with acne. Many of the female patients were on oral contraceptives and these produced the expected changes of hormone levels, notably a raised SHBG. This is further evidence that a low SHBG is not a causative factor in acne, since simple oestrogen-progestagen oral contraceptives, despite their effect on SHBG, are known to be without effect on acne.

We accept that some of the previous studies have been on older females, sometimes with late-onset or persistent acne, where different factors may operate. However, the prevalence of acne is still over 50% at age 30,⁵ so our arguments about control groups and selection of patients are still relevant although quantitatively less marked.

To conclude, there is little evidence that high levels of androgens are responsible for some young people having particularly severe acne. Circulating androgens play, at the most, a permissive role in acne. Much of the published clinical evidence is based upon an unsound experimental approach that fails to recognize the consequences of the high prevalence of acne.

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Two novel techniques for the evaluation of barrier creams

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SUMMARY

There are few methods for assessment of the efficacy of barrier cream preparations that can be used *in vivo*. This paper describes two such tests. The first relies on the ability of a topically applied barrier preparation to prevent dyes from reaching the stratum corneum from filter paper discs in small aluminium chambers. The amount of dye crossing the barrier cream and reaching the stratum corneum was assessed by removing the superficial layers by skin surface biopsy and eluting out the dye. The second test employed dilutions of sodium hydroxide as indicator reagents. The degree to which these solutions penetrated the barrier on the skin was assessed by measuring the skin blood-flow using the laser Doppler method. It was concluded that both methods could be usefully employed to assess the degree of efficacy of barrier cream preparations.

Barrier creams would appear to be a logical approach to the problem of protection of the skin against chemical and physical traumata although they are not accepted by all as being important in the prevention of dermatitis.^{1,2} They are used by large numbers of individuals both in industry and the home, and it seems not unreasonable that evidence should be provided that these products are capable of giving protection in the way suggested by their manufacturers. The lack of evidence of efficacy of barrier creams is in part due to the dearth of suitable standardized techniques for their evaluation and perhaps in part due also to the inadequate barrier properties of the available preparations. In any event it would seem sensible to have suitable methods available that can characterize their properties. Two such techniques are proposed in this paper.

METHODS

Dye exclusion technique

Fifteen normal, healthy volunteers (12 female, 3 male, mean age 31 years, age range 25–38 years) gave their signed, witnessed informed consent to participate in this study. Two barrier preparations were tested in this experiment, both supplied by Stiefel (U.K.) Ltd, Wooburn Green, High Wycombe, Bucks, U.K. One was designed as a water barrier cream (Securiderm Water Barrier, which is dimethicone based) and the other designed as a barrier agent against lipid solvent materials (Securiderm Solvent, which is cellulose gum based). The skin sites tested were delineated rectangles 15 cm × 7 cm on the flexor aspects of either forearm. The two preparations were allocated at random to the forearms of five volunteer subjects in three groups, and 0.5 g of one of the preparations was rubbed into each of the sites until no surplus material was visible on the skin surface. Each group of five subjects were tested with a separate dye. Each of the delineated areas was then subdivided into five sites and four were each treated with one of the following: 0.1% eosin in water, 0.1% methyl violet 2B in water, or 0.5% oil red O in ethanol. The dyes were applied to the skin after being soaked on to filter papers (45 μ l of the aqueous dyes and 10 μ l of the oil red in ethanol) which were then covered by 12 mm diameter aluminium chambers.

The four dye-treated sites were sampled after 5, 15, 30 and 60 min respectively. The fifth barrier cream treated site was not treated with dye but was also sampled at 60 min. A sixth adjoining site, not pretreated with barrier cream, was subjected to dye contact and sampled at 60 min.

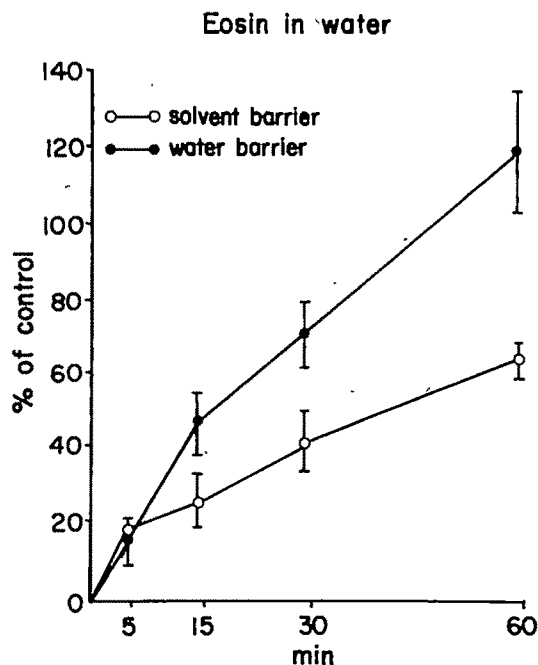


FIGURE 1. The staining of the stratum corneum with 0.1% eosin in water in the presence of a water barrier cream or a solvent barrier cream. Results are expressed as percentage of control $\pm$ standard error of the mean.

Sampling from the five barrier cream treated sites and the adjoining non-barrier cream treated site was performed using the skin surface biopsy technique.³ In order to determine the degree of staining of the stratum corneum at the test sites with the particular dye employed, the skin biopsy from each site was eluted with dimethyl sulphoxide. The optical density of each eluate was then determined at the absorption maximum for each of the dyes using a double beam grating spectrophotometer.

The results from each site were expressed as a percentage of the optical density of the control sites (the unprotected sites exposed to the dye for 60 min).

Alkali irritation method

Eight normal healthy male volunteer subjects (mean age 38 ± 12.8 years) who had given their written, witnessed informed consent, were used in this part of the study. In each subject three marked circular areas, 2 cm in diameter, on the right mid forearm were lightly smeared with a barrier cream (Siopel, ICI Ltd, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.); corresponding sites were marked on the left forearm but received no barrier cream treatment. Thirty min later seven open plastic chambers (with an internal diameter of 12 mm) were fixed on to these marked areas using cyanoacrylate glue—three chambers on the right forearm and 4 chambers on the left forearm. The areas were marked (1) to (4), and treated as follows: site (1) received 4% aqueous NaOH; site (2) received 2% aqueous NaOH; site (3) received 1% aqueous NaOH; and site (4) received no treatment. 100 μ l of each of the alkali solutions were pipetted into each chamber for a period of 15 min after which the forearm skin was carefully dried.

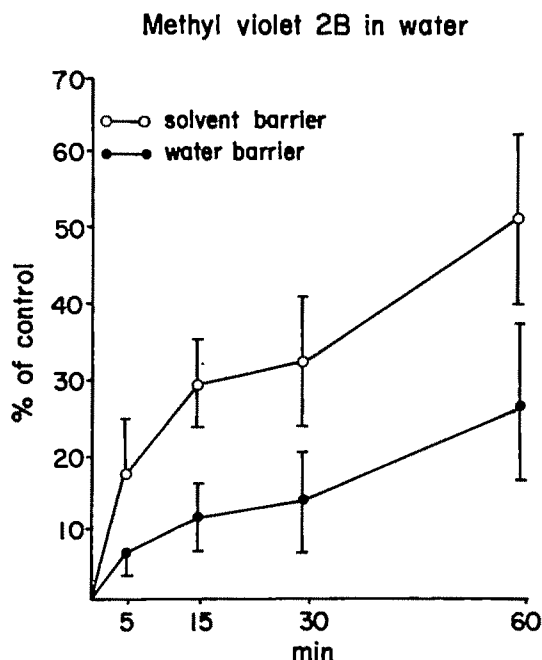


FIGURE 2. The staining of the stratum corneum with 0.1% methyl violet 2B in water in the presence of a water barrier cream or a solvent barrier cream. Results are expressed as percentage of control $\pm$ standard error of the mean.

Dermal blood-flow was recorded from all tested sites at 30, 60 and 90 min after the exposure to the irritant solutions (a total of seven repeated recordings for each time point) using a laser Doppler device (Perimed) to record cutaneous blood-flow in a draught-free room.

RESULTS

Dye exclusion tests

The results for 0.1% eosin in water, 0.1% methyl violet 2B in water and 0.5% oil red O in ethanol are presented in Figures 1, 2 and 3 respectively. It is apparent that there is some degree of protection in each of the experiments as indicated by reduced levels of dye binding to the stratum corneum. Thus, in the experiment where 0.1% eosin in water is applied to the skin, the solvent barrier cream protected the skin such that at 60 min the degree of staining was only 66% of the control site. On the other hand, the water barrier showed no protection at this time. In the experiment where 0.1% methyl violet 2B in water was used, both barrier creams showed protection at 60 min as indicated by reduced dye binding. Thus, the values for the water barrier cream were 27% of control and the values for the solvent barrier cream were 52% of control. In the experiment using 0.5% oil red O in ethanol, the water barrier cream offered protection as indicated by 59% of the dye binding of the control. The solvent barrier cream showed no protection at this time.

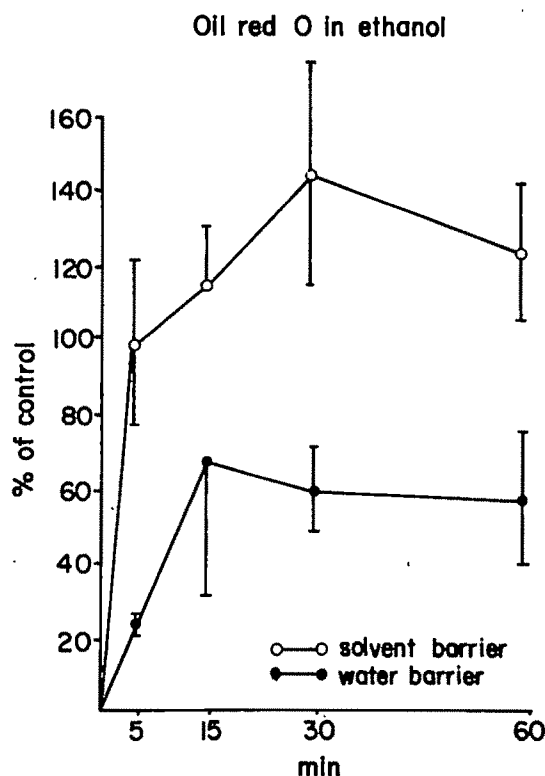


FIGURE 3. The staining of the stratum corneum with 0.5% oil red O in ethanol in the presence of a water barrier cream or a solvent barrier cream. Results are expressed as percentage of control $\pm$ standard error of the mean.

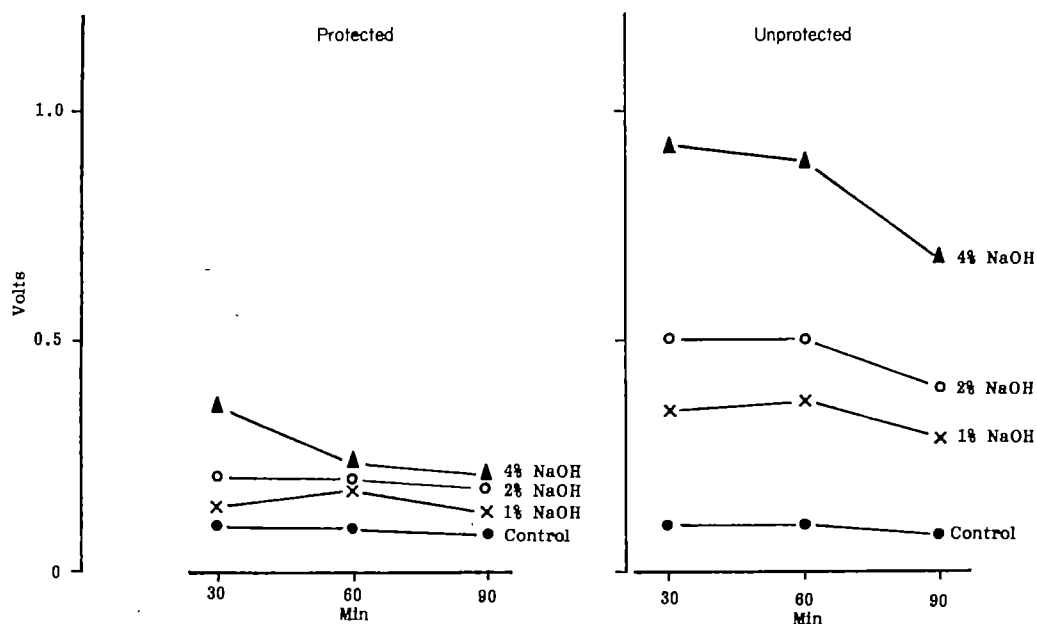


FIGURE 4. Measurement of blood-flow by laser Doppler after application of 1%, 2% and 4% NaOH to sites either unprotected or protected with barrier cream (Siopel). Results are expressed as the mean of eight subjects.

Alkali irritation method

The results obtained using the laser Doppler blood-flow recording device are illustrated in Figure 4. The results show that in the unprotected site there was increasing blood-flow with increased concentration of sodium hydroxide. The increase in blood-flow at the protected sites was much less, indicating that the barrier preparation limited contact with the sodium hydroxide.

DISCUSSION

There are few reports of studies to assess the efficacy of barrier cream preparations. In this study we have used two new techniques that we believe are capable of demonstrating the efficacy of such preparations. In the dye exclusion method the choice of dyes and solvents used for tests with particular preparations influences the results obtained. Thus, the results for eosin in water are the opposite of methyl violet 2B in water with regard to the efficacy of the two barrier creams tested. In this situation it is not simply the ability of the barrier creams to keep out water which is being measured. No doubt the affinity of the dye for the barrier cream and the stratum corneum is important and this may explain the differences observed. Similarly, the poor performance of the solvent barrier compared with the water barrier in keeping out oil red O in ethanol may be the result of the affinity of the dye rather than simply a difference due to degree of exclusion of ethanol. Indeed the values for the solvent barrier in this situation were greater than the controls, suggesting some enhanced penetration of the dye in the presence of the barrier cream.

The tests performed in this part of the study do demonstrate that both barrier creams inhibited contact of the skin with the dyes used in a reverse time-dependent manner. It may be

assumed that these creams prevented contact with the solvents of the dyes in a similar fashion. The increased stratum corneum staining with time indicates that neither barrier preparation is an absolute block to the solvents.

The alkali irritation method used was also successful in demonstrating the protective effects of barrier preparations. Although easier to perform it does require the use of a laser Doppler device and can only supply information on the protectivity of the preparation against penetration by irritating alkali solutions. Nonetheless, this test is easy to perform and may be a useful screening test for the selection of barrier preparations.

Mahmoud *et al.*⁴ used several irritants, including sodium hydroxide and sodium lauryl sulphate, to evaluate the barrier properties of a new preparation in preventing damage to the skin of guinea-pigs evaluated histologically. We believe that it is more appropriate to use human skin and that the histological evaluation is time-consuming and not easily quantifiable. Another technique employing animals was described by Boman *et al.*⁵ in which blood was analysed by GLC after areas of skin were treated with solvents either with or without prior treatment by barrier preparations. This method also has difficulties as not only are the techniques complex and time-consuming but the ability of the solvent to cross the cutaneous barrier is also evaluated.

Recently a test has been described in which excised hairless mouse skin was employed.⁶ Essentially, the test involved mounting the skin in a specially constructed diffusion cell. On one side of the skin was placed (with or without barrier cream) either detergent or sodium lauryl sulphate, and the receptor compartment was filled with physiological saline. The efficacy of the barrier preparation was judged by measuring the electrical conductivity as a function of time post exposure to the irritating challenge material.

Blanken *et al.*⁷ have described a technique to assess the efficacy of barrier preparations in preventing allergic contact dermatitis in individuals sensitized to epoxy resins. This technique may well be of considerable value to evaluate whether particular sensitized individuals can be protected against particular allergens with particular barrier preparations. However, it is unlikely to find wide acceptance because of the need for sensitized individuals.

In conclusion, no previously described technique has emerged as being suitable for the assessment of barrier cream preparations in man *in vivo*. We believe that the two methods described in this paper are relatively simple, safe and cheap to perform and may well be appropriate for industrial use.

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A double-blind study of topical 1 α ,25-dihydroxyvitamin D₃ in psoriasis

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SUMMARY

Several open studies with active forms of vitamin D₃ have been reported to be effective in the treatment of psoriasis. We report a double-blind study of 1 α ,25-dihydroxycholecalciferol in psoriasis using 0.5 μ g of active substance applied topically twice daily. In contrast to previous studies, the present investigation does not provide evidence of clinical efficacy for the drug at that dosage and with the vehicle that was used.

Active forms of vitamin D₃ have been shown to be of potential value for the treatment of psoriasis. Morimoto and Kuhmahara observed a remarkable improvement of psoriasis in a patient who was treated with 1 α ,hydroxyvitamin D₃ for osteoporosis.¹ Two open studies, conducted in Japan,^{2,3} and a recent one from the United States⁴ demonstrated the antipsoriatic potential of active forms of vitamin D₃. These studies showed that 1 α ,25-dihydroxycholecalciferol (1,25-(OH)₂-D₃) was effective on systemic and topical administration^{2,4} as was the application of the systemic analogue 1 α ,24-dihydroxycholecalciferol to psoriatic lesions.⁴ In a recent double-blind study it has been shown that another vitamin D₃ analogue, MC903, improves psoriasis in a dose-dependent fashion.⁵

In this communication we report a double-blind placebo-controlled study on the efficacy of 1,25-(OH)₂-D₃ in the treatment of psoriasis.

METHODS

A total of 10 patients with psoriasis (three female and seven male, aged 28–72 years) participated in the study having given informed consent. All had chronic plaque psoriasis of many years duration. No patient had noticed the appearance of new lesions during the previous 2 weeks before the start of therapy. The extent of the skin lesions varied from two palm-sized lesions up to 15% of the body surface area. At least 2 weeks before the trial, all topical therapies were

discontinued and no patient was on systemic antipsoriatic therapy for at least a month before treatment.

Pregnant women, nursing mothers and women of child-bearing age, not on effective methods of contraception, were excluded from the trial as were patients with internal diseases and concomitant medications. The 24 h-urinary calcium level was measured and routine haematological and clinical chemistry investigations carried out that included determinations of serum calcium, phosphate and parathyroid hormone levels.

The study design was a double-blind randomized intra-patient comparison within each patient, two similar plaques of psoriasis being selected for treatment. Two formulations were given to every patient; one containing 2 µg of 1,25-(OH)₂-D₃ (Ro21-5535, Hoffmann-La Roche, Basel, Switzerland) in 1 ml of medium chain triglyceride and the other the vehicle without 1,25-(OH)₂-D₃. The vehicle was chosen because penetration studies of 1,25-(OH)₂-D₃ in guinea-pig skin had shown that the bioavailability of the drug in medium chain triglyceride was comparable to a cream formulation⁶ and because it was thought to be suitable for the treatment of psoriatic lesions. The bottles containing the test substances were labelled with a code indicating which area should receive the specific solution. For each patient, the allocation of drug or placebo to the specific psoriatic lesions had been randomized and the code was not available to the investigator until after completion of the trial. An aliquot of 0.25 ml of the solution containing 0.5 µg of the active substance was applied twice daily to one lesion, using dispenser pipettes. In the same way, an aliquot of 0.25 ml of the vehicle was applied to the other lesion. After application, the lesions were exposed to air for 5 min. No plastic occlusion was used. The applications were continued for a total of 28 days.

At weekly intervals, erythema, thickness and scaling of the lesions was evaluated using a 4-point scale. At different time intervals the severity scores of the 1,25-(OH)₂-D₃ and vehicle-treated lesions were compared using a Wilcoxon ranking test of paired data. At the end of the trial, 24 h-urinary calcium was determined and blood investigations repeated. The investigations before the start and at the end of the study were compared using a Wilcoxon ranking test for paired data.

RESULTS

The severity scores for erythema, thickness and scaling are shown in Figure 1. Treatment with 1,25-(OH)₂-D₃ and treatment with the vehicle alone produced similar results. No statistically significant difference could be shown between both treatments. In some patients, the vehicle with or without 1,25-(OH)₂-D₃ resulted in some improvement, that was most marked for the parameter scaling ($P < 0.05$).

No side-effects were observed in the areas treated with 1,25-(OH)₂-D₃ and the laboratory investigations were all within the normal range. Table 1 summarizes serum calcium, phosphate and parathyroid hormone levels before and after treatment.

DISCUSSION

In the present study, topical application of 1,25-(OH)₂-D₃ was found to be ineffective in the treatment of psoriasis. No side-effects were observed. A slight improvement was noticed after treatment with the vehicle and this was probably due to lubrication of the skin.

Morimoto and co-workers reported on the efficacy of 1,25-(OH)₂-D₃ in petrolatum for the treatment of chronic plaque psoriasis.² Topical application of 1,25-(OH)₂-D₃ in concentrations

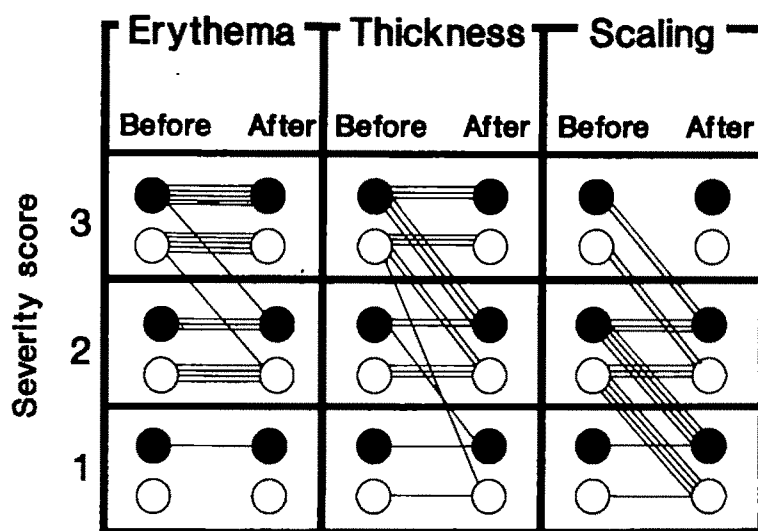


FIGURE 1. Severity scores before and after treatment. ● = lesions treated with 1,25-(OH)₂-D₃. ○ = lesions treated with the vehicle only.

between 0.5 and 1 $\mu\text{g/g}$ once daily yielded excellent clinical results. In another study, there was a marked improvement of psoriatic lesions with the application of 3 μg of 1,25-(OH)₂-D₃ once a day.⁴ Although a relatively high concentration of 2 $\mu\text{g/g}$ was applied twice daily in our study, no clinical efficacy was observed. Plastic occlusion was not used, as in other studies,^{2,4} and this may be a factor. However, excellent results were obtained using topical applications of concentrations of 2 or 4 $\mu\text{g/g}$ of the synthetic derivative 1 α ,24-dihydroxycholecalciferol without plastic occlusion.³ Kragballe *et al.* showed a moderated improvement in patients treated with the vitamin D₃ analogue MC903 in concentrations between 10 and 100 $\mu\text{g/g}$ in water-in-oil emulsion.⁵ He also clearly demonstrated a dose-dependent response with almost no effect with 10 $\mu\text{g/g}$ and an increasing efficacy at 33 and 100 $\mu\text{g/g}$. In the present study, the concentration and frequency of applications of 1,25-(OH)₂-D₃ was thought to be sufficient, but the applications without plastic occlusion might have resulted in insufficient bioavailability of the drug.

In cultures of normal keratinocytes, 1,25-(OH)₂-D₃ has been shown to inhibit substantially epidermal growth.⁷ In animal skin, 1,25-(OH)₂-D₃ also inhibited petrolatum-induced epidermal growth.⁸ On the other hand, pretreatment of normal human skin with 1,25-(OH)₂-D₃ twice

TABLE 1. Serum calcium, phosphate and parathyroid hormone levels and 24 h-urine calcium (mean $\pm$ SD) before and after topical treatment with 1,25-(OH)₂-D₃

	Calcium	Phosphates	Parathyroid hormone	24 h-urine calcium
Before treatment	2.43 $\pm$ 0.08	0.99 $\pm$ 0.15	4.08 $\pm$ 1.00	5.10 $\pm$ 2.83
After treatment	2.41 $\pm$ 0.09	0.89 $\pm$ 0.26	3.83 $\pm$ 0.98	4.98 $\pm$ 2.53

daily at a concentration of 2 µg/g in medium chain triglyceride did not result in an inhibition of the hyperproliferative response to a standardized injury.⁹ In the present study, this same concentration of 2 µg/g was applied without plastic occlusion and yielded negative results.

In conclusion, the present double-blind study of 1,25-(OH)₂-D₃ with concentrations of 2 µg/g, applied twice daily without occlusion, had no clinical effect on the plaques of psoriasis. One possible explanation is insufficient bioavailability of the drug under the present study conditions. Previous studies showing the efficacy of 1,25-(OH)₂-D₃ were performed using a petrolatum vehicle and since this agent stimulates epidermal growth,⁷ an alternative explanation might be that this vehicle changes the epidermal cells so that they can respond to the vitamin D₃.

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Combined treatment of psoriasis with acitretin and UVB phototherapy compared with acitretin alone and UVB alone

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SUMMARY

The administration of UVB phototherapy and low-dose acitretin (0.34–0.44 mg/kg body weight) was compared with the effect of acitretin alone and UVB phototherapy alone in 41 patients with plaque-type psoriasis. Of these patients, 32 received standard UVB phototherapy without acitretin. The other nine were treated with acitretin and the effect of UVB irradiation (Sylvania UV 21-tubes), applied to one half of the body, was assessed. Clearance was defined as 80–100% improvement and this occurred in eight out of the nine patients (89%) treated with acitretin-UVB (ReUVB) and, in two of them (23%), on the untreated side. Clearance occurred in 20 of the 32 (62.5%) patients given UVB alone. The improvement score was significantly higher for the ReUVB side than the acitretin side. Patients treated with ReUVB showed a statistically higher therapeutic score (95–100% clearance) than those receiving UVB alone. However, taking 80–100% improvement as the criterion, no significant difference was found. The number of treatments to clearance was significantly less for the patients treated with ReUVB than for the UVB (19.3 as compared with 24.9). The total UVB dose and the number of minimal erythema doses (MEDs) could be reduced by approximately 20% in the ReUVB group relative to the UVB group.

Many treatments have been used for psoriasis, including etretinate (Ro 10-9359). Initially this was thought to be a potent and non-toxic drug that was effective as a single agent for the complete and permanent suppression of psoriasis. Now it is unlikely that etretinate could be recommended as mono-therapy for plaque-type psoriasis.^{1,2} Etretinate can be useful in the treatment of psoriasis in combination with UV radiation.^{3,4} The combination of phototherapy with retinoids (etretinate) has some important advantages in that it increases therapeutic efficacy and mitigates the long-term hazards of UV exposure by reducing the total UV dose.^{1,2,5}

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The major disadvantage of etretinate was the slow elimination of the drug, the half-life being 84 to 168 days. This is due to the drug being stored in adipose tissue.⁶ The main derivative of the ethylester etretinate is its hydrolysis product acitretin (Ro 10-1670) and this has a shorter half-life, being eliminated in 2 to 4 days.⁶ Earlier clinical trials have shown that the therapeutic effects of acitretin are similar to that of etretinate.^{7,8,9} The aim of the present study was to compare the efficacy of combined acitretin and UVB phototherapy with that of acitretin alone and UVB alone.

METHODS

Patients

Forty-one patients with chronic plaque psoriasis attending the out-patient clinic were included in this study. These were assigned to one of two regimens; acitretin plus half-body UVB radiation (nine-patients), after informed consent was obtained, and standard total UVB exposure without acitretin (32 patients). The details of the patients at the start of the study are shown in Table 1. Patients included in the study had more than 10% of the body surface involved in psoriasis; they had discontinued drugs known to affect psoriasis a month before treatment and were not applying any preparation, apart from 2% salicylic acid in white petroleum. Females of child bearing age were excluded from taking acitretin.

Regimens

Combined regimen. This consisted of a bilateral comparison of an acitretin regimen with and without standard UVB radiation (nine patients). The choice of which half of the body was to be irradiated was made at random. The combined acitretin plus UVB regimen will be referred to as ReUVB.

Acitretin. The drug was administered orally to the nine patients at a daily dose of 30 mg for 2 weeks before the start of the half-body UVB therapy and during treatment. The mean daily acitretin dosage in relation to body weight was 0.39 mg/kg (range: 0.34 to 0.44 mg/kg).

UVB regimen. The ReUVB and UVB groups (9 and 32 patients, respectively) were treated similarly with suberythema doses of UVB. All patients received the first dose of between 30–50% of their minimal erythema dose (MED). With the second treatment the dose increment was given according to a standard schedule;¹⁰ however, the arms and legs did not receive additional

TABLE 1. ReUVB vs. UVB; population characteristics

	ReUVB (n = 9)	UVB (n = 32)
No. of males	7	19
Mean age, year $\pm$ SD	43.7 $\pm$ 14.5	42.0 $\pm$ 14.4
Age range, year	20–64	13–69
Mean duration of psoriasis, year $\pm$ SD	12.9 $\pm$ 11.6	16.9 $\pm$ 12.4
Mean extent of psoriasis $\pm$ SD (% body surface)	17.2 $\pm$ 8.7	16.1 $\pm$ 7.0
Mean minimal erythema dose (MED) $\pm$ SD (mJoules/cm ²)	146.7 $\pm$ 44.0	137.3 $\pm$ 52.9

ReUVB indicates acitretin plus UVB.

UV exposure. After the 23rd and subsequent treatment, the dose of UVB was held constant. If any area of skin became red and sore, the next irradiation was omitted. If only mild symptoms occurred then the preceding dose of UVB was used. Patients were allowed to miss, at the most, one treatment in any 2-week period.

For both groups, the endpoint was taken as clearance of the psoriasis or no further improvement in two successive weeks after a maximum of 30 UVB exposures.

Evaluation of results (ReUVB and UVB groups)

The patients were evaluated before treatment and at two-weekly intervals. The two sides of the body were compared and evaluated and the lesions plotted and graded according to a system used in earlier trials.^{11,12} Scaling and induration were graded from 0 (none) to 8 (very marked), and these scores were used to determine the percentage of overall improvement relative to the patient's status on entry into the study. The therapeutic effect was graded as follows: 0 = 0% to 24%; +1 = 25% to 49%; +2 = 50% to 79%; +3 = 80% to 95%; and +4 = >95% clearance of lesions.

In the nine patients on the combined ReUVB regimen, blood chemistry studies, complete blood cell counts, and urinalysis were done at the start of the study, after 1 month, and at the end of the study. Several of these laboratory investigation (ESR, liver function tests, cholesterol and triglyceride determinations) were repeated after 2 and 8 weeks of treatment.

The Chi-square test, Student's *t*-test and the Signed Rank test were used for statistical analysis.

Light source

The UVB source was a Waldmann UV-1000 cabin with 26 Sylvania F 75/85 W UV-21 fluorescent lamps. Ultraviolet B spectral irradiance measurements were made with a Radiometer with a WBS 320 sensor (International light, Inc., Dexter Industrial Green, Newbury, MA, U.S.A. model IL700). The intensity of wavelengths in the 280–320 nm band at a distance of 20 cm was 1.56 mW/cm². The spectral distribution of the UV-21 lamps and the method used for light-dose control has been described elsewhere.¹²

TABLE 2. Acitretin plus UVB (ReUVB) vs. acitretin alone; results of clearing treatment

Patient no.	Extent of psoriasis at entry (% body-surface)	No. of treatments	Total UVB dose (J/cm ²)	No. of MEDs to clearing	Grading* (0 to +4)	
					ReUVB	Acitretin
1	10	15	6.1	32	+4	+3
2	30	21	9.4	97	+4	+3
3	15	15	8.2	42	+4	+2
4	25	23	16.5	86	+3	+2
5	10	22	8.7	72	+4	+1
6	10	24	8.0	84	+3	+2
7	30	16	3.8	32	+4	+2
8	10	18	6.5	54	+4	+2
9†	15	12	7.8	26	+1	0

MED = minimal erythema dose; Acitretin: daily dose 30 mg.

**P* = 0.012; signed rank test.

†Dropped out of treatment (combination of treatment failure and side effects).

RESULTS

There was no significant difference between the ReUVB and UVB alone groups at the start of the study. The severity of their psoriasis and the MED values were similar in the two groups (Table 1). The results of treatment of the patients for the left-right comparison between ReUVB and acitretin alone are shown in Table 2. One patient stopped treatment after 12 irradiations because of being unable to tolerate the adverse effects of acitretin. Seven out of nine patients (78%) received a score of +2 or +3 for the side treated with acitretin alone, with a clearance of 50% to 95%. On the ReUVB side, clearance (grades +3 and +4) was achieved in eight out of nine patients (89%) with a mean number of 19 irradiations as against two out of nine patients (23%) on the side given acitretin alone in the same period (mean 6.3 weeks). However, acitretin therapy alone never gave complete clearance in any of the patients. The highest score obtained was +3 in two patients (Nos. 1 and 2). Two other patients (Nos. 4 and 6) received a +3 for the ReUVB side and +2 for the acitretin side after 23 and 24 UVB irradiations, respectively, but after that neither side showed any further improvement in either of them. After further treatments, up to 30 UV exposures, their scores were still exactly the same.

There was always a difference of at least one grade between the ReUVB side and the side treated with acitretin alone. A score was available for each side (ReUVB vs. acitretin). Analysis of the paired scores over eight patients showed a significant difference in favour of the ReUVB side (signed rank test, $P=0.012$). Table 2 also shows the individual data on extent of the psoriasis, total UVB dosage, and MEDs to clearing; the mean values of these three parameters are given in Table 3.

Side-effects occurred in all nine patients on acitretin but were generally mild. Cheilitis occurred in all nine and several patients complained of a stuffy nose, nose bleeds, itchy eyes and, in one patient each, there was hair loss, a decrease in libido and, in another, depression. In two patients, the plaques of psoriasis enlarged and became more inflamed during the first 2 weeks of treatment with acitretin. A substantial improvement occurred in one patient with psoriatic nail dystrophy. All patients had normal baseline values of serum cholesterol and triglycerides. In one patient there was an elevation of cholesterol. Of the six patients who developed altered triglyceride levels, four had high and two had low levels. All these changes during the trial were temporary and reverted to normal. There was no reason to withdraw acitretin in any of the cases before the end of the study.

TABLE 3. ReUVB vs. UVB; treatment outcome and dosimetry

Outcome	ReUVB (<i>n</i> =9)	UVB (<i>n</i> =32)
No. cleared	8 (88.9%)	20 (62.5%)
No. of treatments for clearing*	19.3 ± 3.7†	24.9 ± 5.8†
Dose at first treatment* (mJ/cm ²)	65.7 ± 24.5	63.4 ± 37.5
Dose at final treatment* (mJ/cm ²)	847 ± 279	636 ± 282
No. of MEDs at final treatment*	6.2 ± 2.0	4.8 ± 1.8
Total UVB dose to clearing* (J/cm ²)	8.4 ± 3.7	11.1 ± 6.4
No. of MEDs to clearing*	62.4 ± 25.8	78.8 ± 31.6

ReUVB indicates acitretin plus UVB.

* Mean ± Standard deviation; MED: Minimal erythema dose.

† $P < 0.01$, Student's *t*-test.

TABLE 4. ReUVB vs. UVB; improvement scores at the end of the clearing treatment

Response of psoriasis %	ReUVB, No. (%) (n=9)	UVB, No. (%) (n=32)
96-100	6 (67)	6 (18.8)
80-95	2 (22)	14 (43.7)
50-79	—	8 (25)
0-49	1 (11)	4 (12.5)

$P=0.035$, Chi-square test.

ReUVB indicates acitretin plus UVB.

The doses of UVB given to patients in the ReUVB and UVB groups are shown in Table 3. The differences were not statistically significant. The number of irradiations in the ReUVB group was significantly lower than that in the UVB group ($P < 0.01$, Student's *t*-test). The proportion of cleared patients (80-100% improvement) differed widely but not significantly between the two groups, i.e., 89% (8/9) for the ReUVB group compared with 62.5% (20/32) for the UVB group (Table 3). If, however, the psoriatic response is further subdivided into four groups (Table 4), the therapeutic score for the ReUVB group is significantly higher than that for the UVB group ($P = 0.035$, Chi-square test). It appears that the majority of the patients treated with ReUVB scored the highest grade of improvement (95-100% clearance of lesions). In contrast, the majority of UVB treated patients scored one grade lower (80-94% clearance of lesions). If the psoriasis response is divided into three groups (0-79%, 80-95%, and 96-100%), the difference between the therapeutic scores of the two groups is more pronounced ($P = 0.019$, Chi-square test).

DISCUSSION

The oral retinoid etretinate has been reported to be an effective agent in combination with UVB phototherapy.^{3,4,5} This drug has a major drawback in that its terminal elimination half-life is very long because of storage in adipose tissue.⁶ This means that, because of the teratogenic effect of etretinate, pregnancy has to be avoided for at least 2 years after stopping the drug. Acitretin, the hydrolysis product of etretinate, has a terminal elimination half-life of only 2-4 days.⁶ Trials using acitretin as a monotherapy for psoriasis showed similar side-effects and the same efficacy^{7,8} as reported for etretinate therapy.

Seven of the nine patients (78%) in our study treated with acitretin alone, had an improvement score of 50% to 95%. This is comparable with the results obtained by Kingston *et al.*,⁷ who reported 50% improvement or more in 18 out of 21 patients (86%). We used a daily acitretin dose of 30 mg, based on a previous trial that found doses higher than 30 mg a day did not given better results and that the side-effects were more severe.⁸

In our study the mucocutaneous side-effects of acitretin were mild, and there were few abnormal laboratory values. This is in agreement with other studies.^{7,8} In general, the UVB exposures applied in the ReUVB group were well tolerated.

The combination of acitretin and UVB phototherapy was markedly more effective than UVB phototherapy alone. Clearance (80-100% improvement) amounted to 89% for the ReUVB

group compared with 62.5% for UVB alone. ReUVB had therapeutic advantages over UVB. The number of irradiations was significantly lower (19.3 vs. 24.9), which is a mean reduction of 5.6 irradiations or a difference of almost 2 weeks in the duration of the treatment period. The total UVB dose was also lower for the ReUVB group, although this difference is not significant (8.4 vs. 11.1 J/cm²). The results of this trial are similar to those of an earlier trial in which etretinate instead of acitretin was combined with phototherapy for the treatment of psoriasis.¹³

The combination of acitretin and UVB phototherapy is effective during the initial phase of phototherapy. The influence of retinoids on the duration of remission after withdrawal of the drug is still not clear. Continuation of a low dose of retinoid during the maintenance phase does not seem advisable.¹ Perhaps the role of retinoids is limited to the maintenance treatment of patients with severe psoriasis for whom therapy with UVB alone would require excessively frequent exposures of UV radiation.⁴

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Familial keratoses of actinic distribution associated with internal malignancy and cellular hypersensitivity to UVA

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SUMMARY

In members of a family there appeared to be an association between the development of cutaneous pigmented keratoses in sun-exposed sites, and the later evolution of internal malignancies, particularly carcinoma of the uterus. Affected individuals were not clinically photosensitive, but their fibroblasts demonstrated gross cytopathic changes, low survival indices and an increased frequency of DNA single strand breaks following exposure to long-wave ultraviolet radiation (UVA). These clinical and cellular features appear to identify an unrecognized syndrome that may not be uncommon. Recognition of this syndrome in a family may prevent certain malignancies developing in those affected.

After the discovery of a DNA repair defect in xeroderma pigmentosum, patients with excessive and unexplained actinic skin damage have been examined with regard to their cellular response to short wavelengths of ultraviolet light (UVC). These studies have largely provided negative results, with the notable exceptions of Cockayne's and Bloom's syndromes.¹ Much less attention has been paid to the effects of long wavelength UV-radiation (UVA), though such radiation represents a much greater portion of the solar spectrum at the Earth's surface. This probably reflects both a reluctance to look at radiation wavelengths that have been thought to exert their effects only indirectly, through the intermediacy of photosensitizers, and the difficulty of devising appropriate irradiation systems. Recently we have adopted an UVA irradiation procedure whereby the cells are kept in a culture medium that resembles the composition of body fluids. Such a system has permitted the detection of cellular hypersensitivity to UVA irradiation which predisposes to actinic reticuloid²⁻³ and has indicated that the harmful effects of UVA under such conditions are mostly mediated by reactive oxygen species.

We report a family showing with a predisposition to actinic skin damage and cellular hypersensitivity to UVA, apparently associated with a high risk of internal malignancy.

METHODS

Cell strains

Skin biopsies were obtained from nine members of the family (Pedigree nos. IV₃, IV₄, IV₈, V₃, V₄, V₆, V₇, VI₁, VI₂). Fibroblast strains H13457, H13001, H16562, H27118, H15925, H26514, H26515 were established and cultured at 37°C in HEPES buffered Dulbecco's modified Eagle's medium, supplemented with 10% donor calf serum (Flow Laboratories, Irvine, Scotland, U.K.), penicillin and streptomycin. Control fibroblasts were provided by normal individuals of both sexes (H3725, H9172, H15161, H18464, males; H9527, H18800, H20023, H24336, females).

Tests of UVA sensitivity

Cell death was monitored by microscopic observation of cytopathic changes, and by the failure to recover normal levels of ³H-hypoxanthine uptake into RNA. DNA damage was measured after exposure to an UVA sun lamp (320–400 nm) by the determination of single strand breaks as previously described.³ For the measurement of ³H-hypoxanthine uptake and the observation of cytopathic changes, cells were grown to confluency in 35 mm plastic Petri dishes transparent to wavelengths longer than 295 nm and irradiated from above with 60–150 kJ/m² of UVR from a blue-black fluorescent light tube (BLB; Sylvania mostly 320–400 nm), at a dose rate of 4 W/m².

After irradiation, the cells were incubated in fresh medium for a further 24 h, then labelled for 3 h with ³H-hypoxanthine (1 µCi/ml; sp. act. 1.5 Ci/mmol), and finally washed with phosphate buffered saline and fixed with methanol. Cytopathic effects were sought after Giemsa staining. ³H-hypoxanthine uptake by the irradiated cells was measured by liquid scintillation and divided by the value for non-irradiated cells, so as to provide a 'survival index' (SI) with a maximum value of 1 (SI-³H-hypoxanthine in irradiated cells/³H-hypoxanthine in non irradiated cells).

DNA strand breaks were measured by the alkaline elution technique of Kohn *et al.*,⁴ as follows. Cells, labelled uniformly for 72 h with ³H- or ¹⁴C-thymidine (0.1 µCi/ml; sp. act. 0.1 Ci/mmol; and 0.01 µCi/ml; sp. act. 60 mCi/mmol, respectively) were incubated for 48 h in isotope-free medium. They were then placed on a glass/water/glass filter (the temperature of which could be controlled from 4°C to 25°C) and irradiated from below, at a fluence of 50 W/m² with a UVASUN 2000 (Mutzhas Corp. 320–400 nm). After irradiation, the patients' and controls' cells, labelled with different isotopes, were scraped into ice-cold PBS/0.02% EDTA, mixed, lysed on Millipore 2 µm pore-size filters and their DNA eluted through the filter with 0.1 M tetrapropylammonium hydroxide/0.02 M EDTA (free acid form) buffer, pH 12.1, at a flow rate of 0.03–0.04 ml/min. Ten fractions were collected at 90 min intervals and the ³H and ¹⁴C disintegrations determined by liquid scintillations with the help of a computer program. The regression of the labelled DNA retained by the filter on the period of elution was then used to determine the number of DNA single strand breaks per 10⁸ nucleotides, as outlined by Kohn *et al.*⁴

Unscheduled DNA synthesis

This was determined after germicidal UV-radiation (95% 254 nm), by the amount of ³H thymidine taken up by cells exposed to 5 and 15 J/m² and not undergoing DNA replication, as described previously.⁵

DNA daughter strand repair was examined as described by Lehmann *et al.*⁶

Chromosome breakage was determined in peripheral blood lymphocytes by standard procedures.^{7,8}

CASE REPORTS AND EXPERIMENTAL RESULTS

Case reports

The family shown in Figure 1 came to our attention following the report of case 1 (IV 4).

Case 1—(IV 4). A 68-year-old lady first noticed the appearance of skin lesions at the age of 35, during a particularly hot, sunny summer. The initial lesions were flat, pigmented and

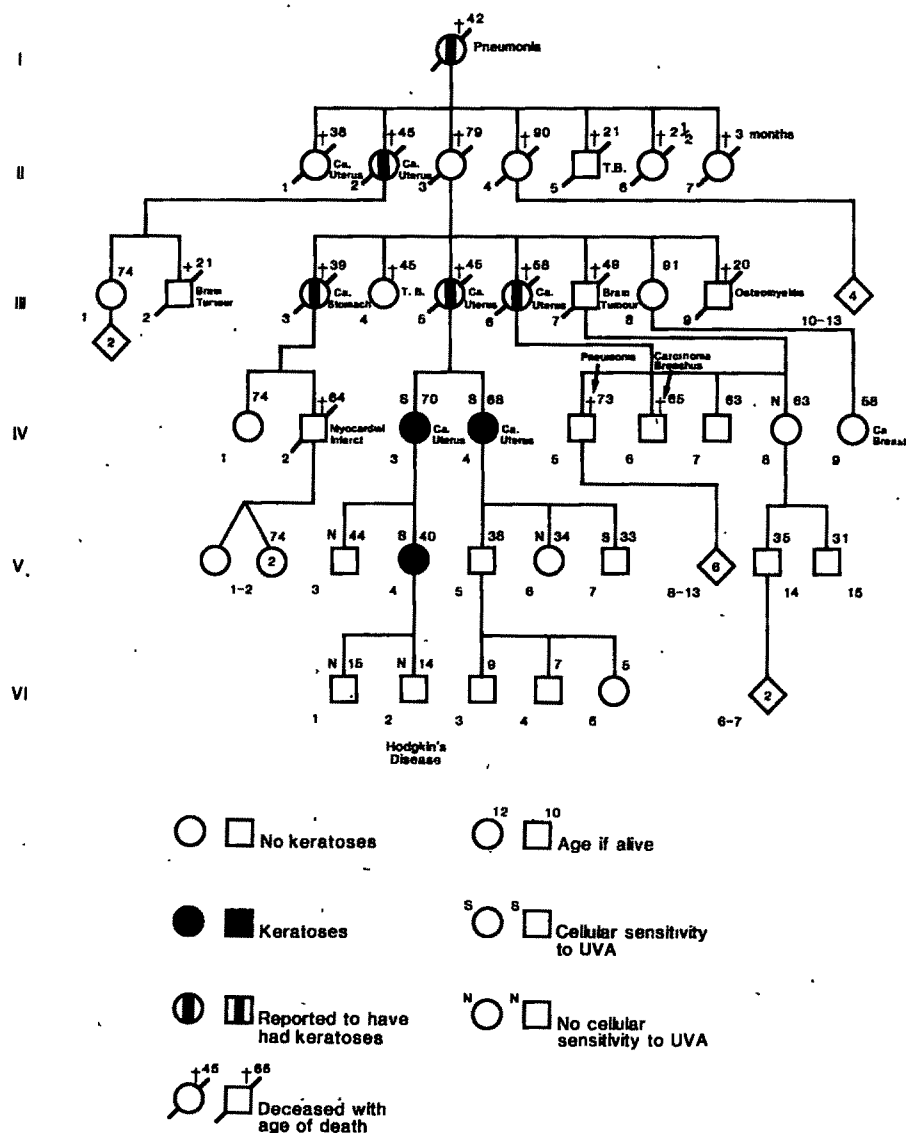


FIGURE 1. Pedigree of the family. ◇ indicates numbers of offspring of both sexes.

asymptomatic, and were located on the face and the dorsa of the hands. During the next few years, similar lesions also arose on the extensor aspects of the forearms and lower legs. The lesions increased in number and may have become raised and warty. This lady tans readily and at no time has been aware of undue sensitivity to sunlight, though solar exposure tends to make the lesions itch. She has never lived outside the U.K.

A keratoacanthoma was excised from her face at 59 years, and a basal cell carcinoma at 65 years. A total hysterectomy and left salpingo-oophorectomy for an adenocarcinoma of the uterus were performed when the patient was 43 years old.

On examination the exposed areas of skin appeared excessively aged relative to the reported mild degree of solar exposure. The lesions were four times more numerous on the extensor surface of the forearm and lower legs as compared with the flexor surface. A few lesions were present in the 'V' area of the upper chest, but there were none under the chin. Elsewhere there were only a few lesions. Most were flat and slightly keratotic, of a café-au-lait hue and under 1 cm in diameter (Fig. 2). Some appeared depressed. Others were faintly erythematous, more atrophic and similar to the lesions seen in disseminated superficial actinic porokeratosis, though the distinctive raised edge was lacking. A number were raised and verrucous; these were mostly under 0.5 cm in diameter and closely resembled 'stucco' keratoses.^{9,10} More rarely, they had the form of larger, sessile warts that were indistinguishable from hyperkeratotic seborrhoeic keratoses. Between these various types there were many lesions with intermediate features. The patient was otherwise well. Monochromator light tests performed on this lady were normal.



FIGURE 2. Case 1 (IV4). (a) Face. (b) Dorsum of left hand. Most of the lesions visible are flat and of a light brown colour. Some are mildly hyperkeratotic, but the majority of more verrucous lesions have been excised, or ablated by liquid nitrogen.

Case 2—(IV 3). This patient was a year older than her sister (*Case 1*). She noticed the progressive appearance of pigmented skin lesions from the age of 27 years, at first on the dorsa of the hands and then on the extensor surfaces of the forearms and later on the face. Over the next 10 years they also appeared on the lower legs, and on the flexor aspects of the forearms. After the age of 40, increasing numbers of these lesions became reddened, particularly those on the forearms. Her skin was paler than her sister's and she was more sensitive to sunlight. She did not consider that she was abnormally sensitive to the sun, though she noticed that new lesions tended to appear after significant sun exposure.

At the age of 43 years, she had a right mastectomy for cystic hyperplasia, and had several cysts excised from the left breast. At the age of 45 years, she had total hysterectomy and bilateral salpingo-oophorectomy for an adenocarcinoma of the uterus. At 50 years of age, she developed a peripheral neuropathy that has progressed and she now has parasthesiae and numbness affecting mainly the legs, and weakness that prevents her from walking unaided. So far, no specific cause for her polyneuropathy has been found.

On examination the skin findings were similar, but more marked, to those of her sister. Her skin was pale and perhaps for this reason, the erythema of many of the flat lesions was more prominent. The lesions were also more numerous than in her sister's case and with a greater proportion of raised warty lesions. Their distribution was virtually identical, with lesions more or less confined to the exposed areas of the face (*Fig. 3*), the dorsa of the hands, the forearms and the lower legs. Many more were present on the extensor aspects of the forearms than on the flexor aspects. A few lesions were present on the outer aspects of the upper arms. Monochromator tests were normal.

The principal neurological findings are those of a mixed sensori-motor peripheral neuropathy, with distal muscle weakness in all four limbs, but particularly the legs, with complete areflexia, total absence of vibration sensation and joint position in the limbs, plus 'glove-and-stockings' sensory disturbance.



FIGURE 3. *Case 2 (IV₃).* Right side of the face, showing pigmented and erythematous hyperkeratotic lesions, some resembling 'stucco' keratoses.

Case 3—(V4). This lady is now aged 40 years. During the last 10 years she has developed a few pigmented macules (less than 3 mm in diameter) on the back of the hands. These resembled closely those that were present at about the same age in her mother (Case 2) and her aunt (Case 1). Recently she had a prophylactic total hysterectomy with conservation of the ovaries. The excised uterus appeared normal, both macroscopically and on histological examination.

Histopathology

Numerous skin biopsies have been taken from patients 1 and 2, including several examples of each of the three main types of clinical lesion, i.e. (i) flat or depressed lesions, (ii) domed or flat-topped keratotic papules of stucco keratosis type, and (iii) sessile seborrhoeic wart-like papillomata.

The flat or depressed lesions (type 1) generally show mild and variable hyperkeratosis and acanthosis, with a definite tendency to basal budding (Fig. 4). There was no evidence in any specimen of a cornoid lamella. The lesions showed a variable degree of an associated upper dermal chronic inflammatory cell infiltrate.

The domed or flat-topped keratotic papules (type 2) show marked hyperkeratosis overlying spiky acanthosis (Fig. 5). Some sections showed occasional columns of parakeratosis. In many areas the epidermal cells have a disorganized, 'stirred up' arrangement with zones of keratinization, as seen in 'irritated' seborrhoeic warts. In some lesions, individual cell keratinization was a feature. Basal budding of the epidermis was often present. These papules showed a variable degree of associated upper dermal inflammation. In some lesions, the infiltrate was dense and lichenoid with inflammatory cells within the lower layers of the epidermis and associated colloid bodies.

The sessile papillomata (type 3) showed variable hyperkeratosis and acanthosis with squamous eddies, horn cysts and associated papillomatosis. Basal budding was a feature in some lesions. An upper dermal inflammatory cell infiltrate was invariably present.

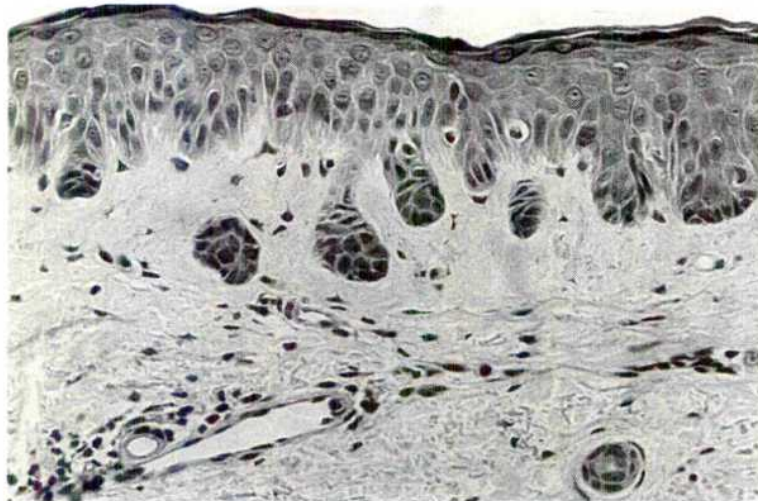


FIGURE 4. High power photomicrograph of a section from a type 1 lesion. The hyperpigmentation and budding of basal cells is clearly seen. (H & E, $\times 96$).

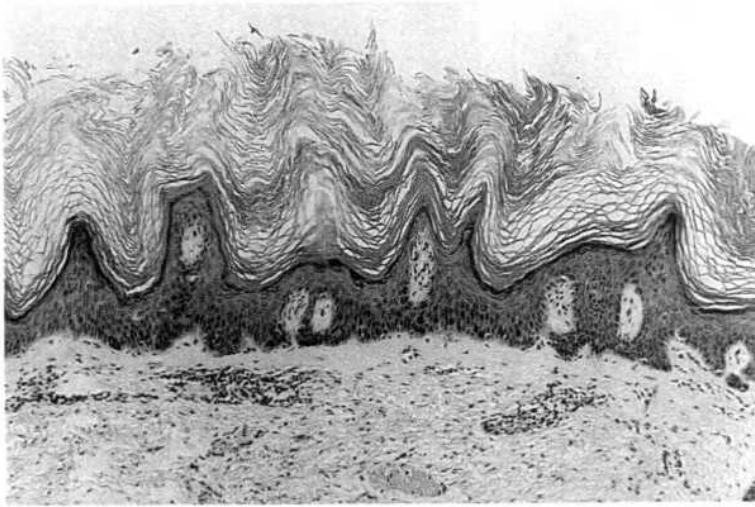


FIGURE 5. Spiky acanthosis and marked hyperkeratosis is visible in this type 2 lesion, resembling a 'stucco' keratosis. (H & E, $\times 36$).

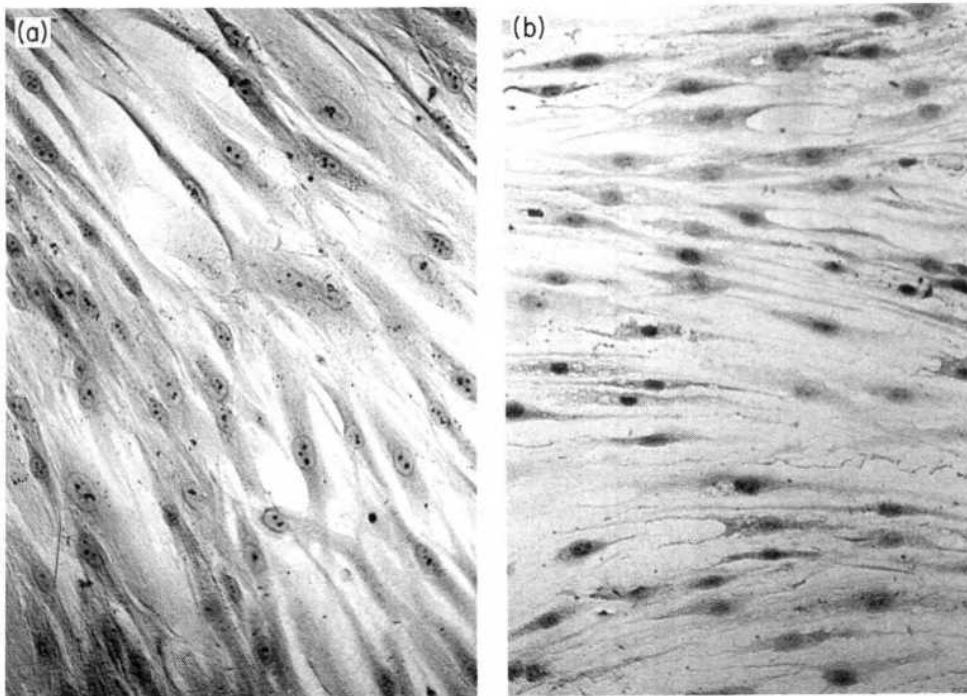


FIGURE 6. (a) Control fibroblasts irradiated with UVA (100 kJ/m^2) and incubated for 24 h. (b) Fibroblasts from IV3 treated as the controls in (a). Note the disappearance of nucleoli, nuclear pyknosis and cytoplasmic damage.

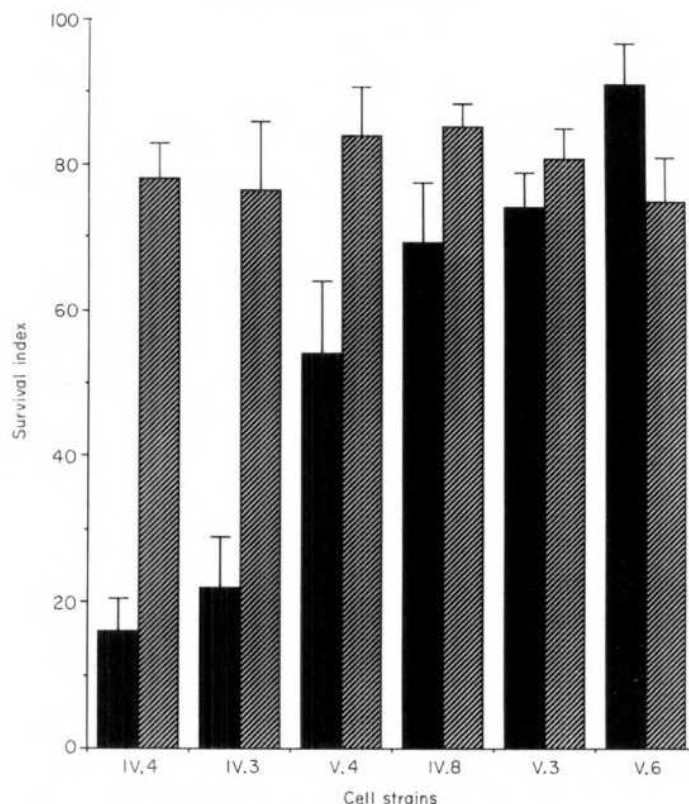


FIGURE 7. The histogram shows the ^3H -hypoxanthine incorporation of cells irradiated with UVA (100 kJ/m^2) relative to non-irradiated cells. Measurements were made 24h after irradiation. Solid bars are data from members of the family under investigation (see Figure 1 for pedigree numbers). Stippled bars are data from co-irradiated cells from normal volunteers. Line at top of the bar indicates $+1 \text{ SE}$.

Genetics

The pedigree of this family (Fig. 1) indicates that the condition may have occurred in several generations, and suggests an autosomal dominant pattern of inheritance. Either the penetrance or expressivity of the responsible gene seems variable and the gender appears to influence manifestation of the disease. A number of members of the family were still too young or had died too young for the condition to have become manifest.

There appears to be an association between the cutaneous pathology and the occurrence of internal malignancies, particularly carcinoma of the uterus. Thus, out of six women with the skin lesions, four had adenocarcinoma of the uterus, one adenocarcinoma of the stomach, and one had died relatively early in life, at the age of 42 years, from pneumonia. Case 2, died at the age of 38 years of carcinoma of the uterus, apparently without demonstrating typical skin lesions. However, in this case, because of her age, it is possible that the lesions were not obvious and were overlooked.

Cell biological and biochemical findings

Long wavelength UVR induced abnormal responses in cells from all the affected members of the family who were tested. These abnormal responses comprised the development of gross

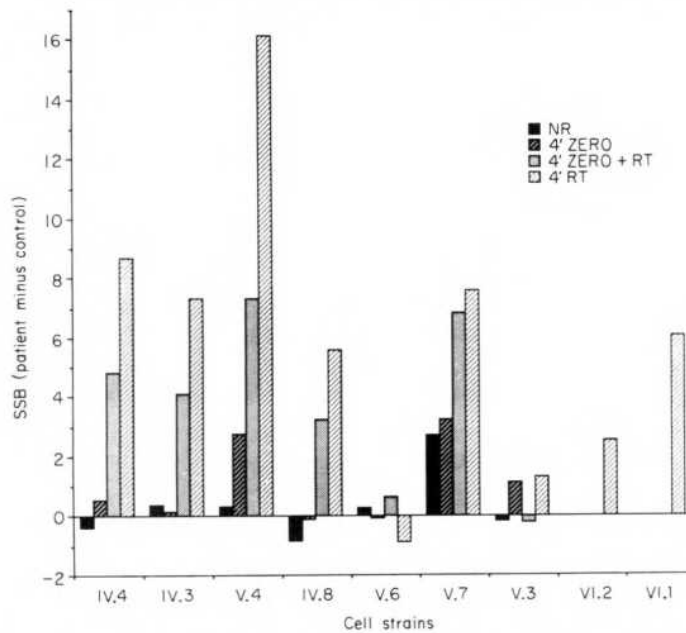


FIGURE 8. The histogram shows the difference between the single strand DNA breaks (SSB) caused by the UVA irradiation (100 kJ/m^2) in test and control cell strains under different experimental conditions. NR = not irradiated. 4'zero = cells irradiated over a 4 min period while kept at $0-4^\circ\text{C}$ and then assayed for SSB. 4'zero + RT = cells irradiated as above but then kept at 23°C for 5 min before assaying SSB. 4'RT = cells irradiated at 23°C for a 4 min period and immediately assayed for SSB. IV4-VI1 = data on individuals from family under study (see Fig. 1 for pedigree numbers).

From left to right, the 4th, 12th and 24th difference is significant at the 0.5% level and the 7th and 8th at the 1% level.

cytopathic changes, low survival indices and an increased frequency of DNA single strand breaks. The cytopathic changes occurred within a 24 h period, following 8–10 h exposure to UVA and included massive cytoplasmic disruption and nuclear pyknosis. Control strains, and those from non-sensitive relatives remained unaffected for up to 12–14 h exposure (Fig. 6). The survival index was abnormal in IV3, IV4, and V4 (Fig. 7).

The number of DNA single strand breaks/ 10^8 nucleotides detected in irradiated cells are shown in Fig. 8. This indicates that the cells of IV3, IV4, V4 and V7 show a clear, statistically significant excess of breaks except when they are irradiated at 4°C and harvested immediately afterwards so as to inhibit the incision of damaged DNA which occurs during repair. The cells from IV8, V3, V6, VI1 and VI2 do not show such a statistically significant excess.

TABLE 1. DNA repair synthesis after UV irradiation (254 nm) in fibroblasts from members the family investigated

Individual	IV3	IV4	IV8	V3	V4	V6	V7	VI1	VI2
DNA repair synthesis as % of normal controls	80	82	95	83	84	90	92	99	85

Unscheduled DNA synthesis after 254 nm UV irradiation was slightly lower than controls in some members of the family but always within the normal range (Table 1).

Daughter strand repair; defective in xeroderma pigmentosum variants, was normal in the three tested UVR sensitive cell strains (IV₃, IV₄ and V₄), and the level of chromosome breakage in peripheral blood lymphocyte cultures of IV₃ and IV₄ was similar to that of controls after X-ray and mitomycin C exposure.

DISCUSSION

We have described a family in which some members developed multiple cutaneous keratoses. The lesions did not show distinctive features either clinically or histologically but were remarkable only because of their great number and their predilection for light exposed areas.

The majority of the skin lesions found in affected members of this family were indistinguishable from the flat pigmented lesions commonly found on the dorsa of the hands in older persons, and popularly known as 'liver spots'. They were, however, numerous and widely distributed. A proportion of the flat lesions were suggestive of those of disseminated superficial actinic porokeratosis,¹¹ though none showed a peripheral ridge. Many of these were erythematous, and some were distinctly atrophic at their centre. These inflamed lesions were sometimes itchy. Histologically, these flat keratoses had features similar to those of disseminated superficial actinic porokeratosis, but lacked a cornoid lamella.

The second type was a domed, pigmented and slightly verrucous lesion that was clinically and histologically indistinguishable from the 'stucco' keratosis.^{9,10} The third type was a sessile pigmented papilloma, clinically and histologically resembling a seborrhoeic wart, but differing in its distribution. Histologically, both types of raised lesions frequently showed a 'stirred up' orientation of the keratinocytes, with focal zones of keratinization and a regular association with reactive dermal inflammation. Such lesions were closely similar to those observed in skin tumours in mice exposed to experimental photochemotherapy (N. Smith—personal communication).

Although the late onset and distribution of these cutaneous lesions suggest that they are induced by solar radiation, they are not associated with abnormal acute responses to sunlight or to monochromatic light of wavelengths between 300 nm and the visible spectrum (I.A. Magnus—personal communication).

Only females appeared to have been affected in this family, and skin lesions were associated with internal malignancy, particularly carcinoma of the uterus. It is difficult, of course, to place absolute confidence in anamnestic data. However, in this family, uterine malignancy and skin lesions show a remarkable degree of association, which was obvious to the patients. The pedigree suggests that the predisposition to actinic skin damage and possibly to internal malignancy was transmitted in a dominant manner.

Our cellular studies were consistent with such an inheritance. They show transmission from mother to daughter of a cellular hypersensitivity to UVA radiation (a situation not expected in rare recessive conditions). Other modes of inheritance are not excluded by such studies. The abnormal cellular sensitivity to UVA radiation, presumably predisposes the excessive actinic damage. Such hypersensitivity to UVA is clearly different from that seen in xeroderma pigmentosum, xeroderma pigmentosum variants, Cockayne's and Bloom's syndrome, where the fibroblasts are sensitive to UVC and UVB but respond normally to UVA. However, it is similar to that seen in actinic reticuloid.^{2,3} In the latter condition there is, however, clear evidence of abnormal cutaneous sensitivity to natural and monochromatic light in the UVA and

even in the visible spectrum. We have found strong circumstantial evidence that in actinic reticuloid the cellular defect impairs the neutralization of oxygen-related free radicals (Butcherby and Giannelli, unpublished). It is tempting to offer a similar explanation for the hypersensitivity to UVA radiation shown by the cells of the patients from the family reported in this paper for the following reasons. There is good evidence that UVA in our system of irradiation acts mainly by the generation of oxygen free radicals. The cytopathic changes develop very rapidly, unlike those caused by shorter UV irradiation and other agents acting directly on the DNA. In fact, the hypothesis of a defect in the mechanisms which dissipate or neutralize oxygen free radicals could explain the association of hypersensitivity to actinic damage in the skin with the apparent high risk of internal malignancies, as there is evidence for the involvement of free radicals at least in the promotion of carcinogenesis.¹²

The clinical and cellular features we have described appear to identify an unrecognized syndrome. Abnormal cellular and skin sensitivity to actinic damage may be associated with internal malignancy in other families. Early recognition of the syndrome may allow the prevention of potentially lethal cancer. Indeed, we considered that the development of characteristic skin lesions, plus the demonstration of abnormal cellular UVA sensitivity in Case 3 (V4) was sufficient to indicate a prophylactic hysterectomy.

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Cutaneous cryptococcosis: treatment with oral fluconazole

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SUMMARY

A case of cutaneous cryptococcosis is described in an immunocompromised patient. The initial lesion developed on the dorsum of the hand following trauma and was initially thought to be neoplastic. Satellite subcutaneous lesions developed in a 'sporotrichoid' pattern along the forearm. Treatment with oral fluconazole resulted in the complete resolution of the lesions. This is the first published report of the use of fluconazole in the treatment of cutaneous cryptococcosis.

Cutaneous cryptococcosis, the result of infection by *Cryptococcus neoformans*, is an uncommon fungal infection in the normal healthy population, but is more common in patients who are immunocompromised by virtue of disease (e.g. underlying malignancy, AIDS) or drugs. Although established treatment with 5-fluorocytosine and amphotericin B is effective, these drugs have to be given intravenously and their use may be limited by toxicity, especially in elderly patients with impaired renal function. The recent development of the triazole group of antifungal agents, such as fluconazole and itraconazole, has led to the possibility of oral treatment for deep mycotic infections such as cryptococcosis.¹ We describe a patient with primary cutaneous cryptococcosis treated with fluconazole.

CASE REPORT

The patient, an 85-year-old man, was referred to the Dermatology Department with an ulcer on the dorsum of the left hand. This had been present for approximately 3 months. The ulcer had developed at the site of a superficial laceration sustained on a door catch in the patient's own home. Over the course of the next few weeks the ulcer was dressed regularly with antiseptic dressings, but it continued to enlarge. Pain was a prominent feature. Two months after the initial injury, the patient developed two tender swellings on the left forearm. These were also

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painful. Over the past 4 years he had been treated for polymyalgia rheumatica with prednisolone, 10 mg/day. Apart from a mild psoriasis, there was no other significant past medical history. Two fingers from each hand had been lost in a childhood accident.

On examination there was an irregular ulcer, 5 × 4 cm in size, on the dorsum of the left hand (Fig. 1a). The edges of the ulcer were elevated and 'rolled', and a neoplasm was initially suspected. In addition, there were two firm, mobile, tender subcutaneous nodules approximately 3 cm in diameter, on the extensor aspect of the left forearm. Apart from mild psoriasis on the trunk, no other abnormalities were found, and he was not systemically unwell.

Investigations

Biopsies of the ulcer and the nodules on the forearm were taken. Histological examination of both sites showed large aggregates of histiocytes with some plasma cells and lymphocytes in the

(a)



(b)

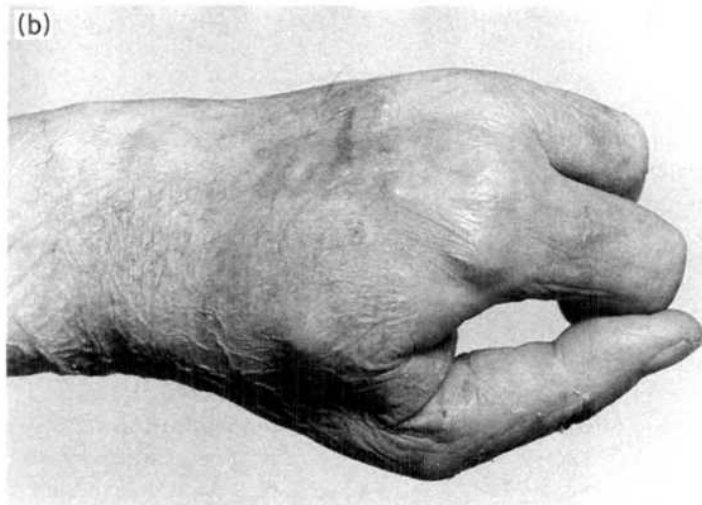


FIGURE 1. Cryptococcal ulcer on dorsum of the hand: (a) before treatment, (b) following treatment with fluconazole. The ulcer has completely healed.

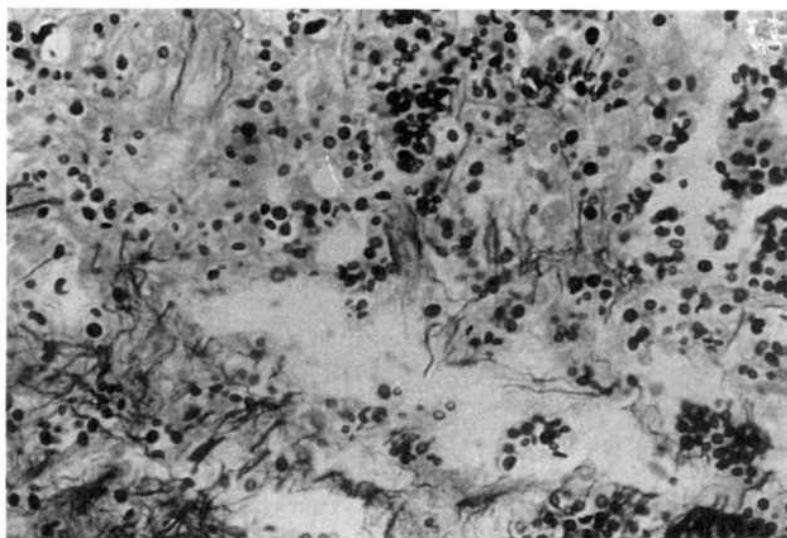


FIGURE 2. Histology of the ulcer. Numerous small round yeasts are seen. (Silver methanamine stain; original magnification $\times 60$.)

dermis. Within the histiocytes there were numerous cytoplasmic inclusions, which stained positively with periodic acid-Schiff and methanamine silver stains and budding was occasionally seen (Fig. 2).

A Gram stain of a squash preparation from fresh biopsy material showed the presence of numerous budding, non-hyphal yeasts with a distinct small capsule. A presumptive identification of *Cryptococcus* was confirmed by isolation of the yeast on 3% malt extract agar with 0.5% gentamicin at 37°C after 48 h. Biochemical typing showed the yeast to be *Cryptococcus neoformans*.

Examination of urine and CSF by Indian ink mount and culture was negative for *Cryptococcus*. The CSF was clear with 0 white cells/mm³, and the pressure was normal. The latex agglutination test for capsular polysaccharide was negative in CSF. Full blood count, liver function tests and chest X-ray were all normal. There was a moderate impairment of renal function, the creatinine clearance being 25 ml/min.

In an attempt to identify the source of the infection, swabs were taken from the door catch and handle in the patient's flat. Swabs were also taken from the window frames and window sills. No yeasts were identified from any of these swabs. Pigeon excreta was not found.

Treatment

Treatment was started with oral fluconazole (Pfizer Ltd, Sandwich, Kent, U.K.), 200 mg daily. Serum levels at 10 days were measured with a standard agar bioassay using *Candida pseudotropicalis* as the indicator organism. Peak and trough levels (15.2 µg/ml and 18.3 µg/ml respectively) were found to be approximately twice those expected in a normal healthy adult. Fluconazole is almost completely excreted by the kidney and these high levels were probably the result of the patient's impaired renal function. The dosage was reduced to 100 mg/day and subsequent trough serum levels fell to approximately 12 µg/ml. During treatment, swabs were taken at intervals from the ulcer on the hand in order to monitor the mycological response. At 13

days, yeasts could still be seen in a Gram stain of the swab and could be cultured (although only after 4 days). Twenty-three days after the start of treatment, yeasts could not be seen on a Gram stain and only three colonies were grown after 6 days incubation. At 39 days, the Gram stain was negative and no growth could be obtained. The ulcer on the dorsum of the hand was completely healed on the 46th day following the start of treatment (Fig. 1b). A total of 8 weeks treatment was given.

Treatment with fluconazole was well tolerated and no adverse reactions occurred, even at the initial dosage of 200 mg daily, when serum levels were high. No biochemical or haematological changes occurred during treatment.

DISCUSSION

Localized cutaneous cryptococcosis is unusual although there are previous reports of its occurrence following superficial injury to the skin.² The resemblance of the lesions to primary skin neoplasms is well recognized.³ Most cases of cryptococcosis in which there is skin involvement arise as the result of secondary spread from systemic infection.⁴ The clear history of trauma preceding the development of the lesion in our patient and the absence of any evidence of systemic infection supports the diagnosis of primary cutaneous cryptococcosis, as does the apparently 'sporotrichoid' spread along the forearm. In the past, primary cutaneous cryptococcosis has been defined on the basis of three criteria: (i) the lesion is confined to the skin and subcutaneous tissues; (ii) the yeast is isolated and seen histologically; (iii) there is no evidence of systemic involvement in the 4 weeks following diagnosis.⁴ There is some controversy as to whether a primary cutaneous chancroidal syndrome, (as seen for example following inoculation with *Histoplasma*), ever occurs following inoculation with *Cryptococcus*. It may be that prolonged follow-up of our patient will reveal evidence of underlying systemic infection. In one report, cutaneous lesions developed 2-8 months prior to the development of CNS infection.⁵ It is worth noting that cutaneous cryptococcosis following inoculation of the organism may be a self-limiting disorder, but the sporotrichoid spread of the infection, together with the increase in the size of the ulcer until treatment was started makes spontaneous resolution unlikely in this case.

It seems likely that the infection was facilitated by the age of the patient and treatment with prednisolone. We were unable to identify a source of infection in our patient although we excluded contamination of the patient's home with pigeon excreta, which is a potential reservoir of infection.⁶ Fluconazole is a new orally active triazole fungistatic agent.⁷ It is well absorbed and relatively non-toxic.⁸ High levels of active drug are obtained in cerebrospinal fluid following oral administration, and there are a number of published reports of its use in cryptococcal meningitis.⁸⁻¹⁰ The use of fluconazole in cutaneous cryptococcosis does not, however, appear to have been previously reported, but the results of treatment in our patient suggest that it may be useful in cutaneous mycoses where current agents are precluded by toxicity.

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Non-symmetric subcutaneous lipomatosis associated with familial combined hyperlipidaemia

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SUMMARY

A family with familial combined hyperlipidaemia in which affected members had non-symmetric subcutaneous lipomatosis (NSSCL) is described. Affected members had high serum levels of total cholesterol, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. By contrast, family members without NSSCL had normal lipid levels. There was also a correlation between the degree of hyperlipidaemia and the amount of subcutaneous lipomas. The occurrence of hyperlipidaemia in family members with NSSCL suggests the existence of a genetic linkage between these two characteristics, but did not show any association with HLA haplotyping.

To our knowledge this association between lipid abnormalities and NSSCL has not been previously reported.

Non-symmetric subcutaneous lipomatosis (NSSCL) is a benign familial disease without any metabolic significance or detectable abnormalities of lipid metabolism. The lipomas consist of normal adipocytes although of a larger size.¹ On the other hand, multiple symmetric lipomatosis (MSL) is characterized by large lipomas in the neck and shoulder region, is also familial and is inherited as an autosomal dominant trait. This condition may be associated with hypertriglyceridaemia, hyperuricaemia and impaired glucose tolerance. It is almost always associated with elevated levels of HDL cholesterol. The cells in the lipomatous tissue are slightly smaller than those of normal adipose tissue and have an enhanced activity of lipoprotein lipase.²

We have recently encountered a family with familial combined hyperlipidaemia, in which affected members also had NSSCL, high serum levels of total LDL- and HDL-cholesterol. By contrast, family members without NSSCL had normal lipid levels. The occurrence of hyperlipidaemia in family members with NSSCL suggests the existence of a genetic link

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between these two characteristics. This family is unique due to the association of NSSCL and abnormalities of lipid metabolism in the affected members. To the best of our knowledge, such an association has not been previously reported.

METHODS

Nine members of a family with NSSCL were studied in the lipid clinic of the Tel-Aviv Medical Center (Table 1, 1–9). Each member had a complete history taken and a physical examination as well as laboratory investigations that included a complete blood count, urine analysis, fasting blood glucose, liver and renal function tests, serum thyroxine and serum uric acid.

Repeated determinations of cholesterol and triglyceride were performed on a 12 h fasting serum; the cholesterol was determined according to the method described by Huang *et al.*³ and triglyceride by an enzymatic method.⁴ HDL cholesterol was determined in the supernatant after precipitation of APO B containing lipoproteins.⁵

HLA typing was performed by the standard NIH two-stage micro-lymphocytotoxicity technique.⁶

RESULTS

The proband was a 55-year-old woman with known hypercholesterolaemia (up to 550 mg/dl) and normal triglyceride levels for more than 25 years. She had NSSCL for more than 30 years with, over the years, an increase in the number of the lipomas. She was otherwise healthy. One lipoma was excised from the axilla because of local discomfort; histological examination revealed normal adipose tissue with adipocytes of various sizes.

Her physical examination and laboratory tests were unremarkable except for NSSCL with lipomas ranging in size from 1–4 cm in diameter, and xanthomata on both Achilles tendons. The laboratory findings were cholesterol of 412 mg/dl and LDL cholesterol of 312 mg/dl (Table 1).

Her oldest son, aged 33 (No. 2) also had multiple NSSCL. His serum cholesterol was 330 mg/dl, and LDL cholesterol 240 mg/dl, with a mild elevation of triglyceride levels (205 mg/dl).

Her youngest daughter, aged 22 (No. 3) had only a few lipomas, total serum cholesterol was 285 mg/dl and the triglyceride was 235 mg/dl. Another daughter, aged 28, had no NSSCL and her serum lipids were in the normal range.

Two of the proband's siblings (Nos. 6 and 9) had hypercholesterolaemia: 319 mg/dl and 305 mg/dl, respectively. One of them, No. 6, also had hypertriglyceridaemia (274 mg/dl), and had a myocardial infarction 3 years previously. He died suddenly during the follow-up period. The other brother (No. 9) had also suffered a myocardial infarction 4 years earlier, and subsequently underwent coronary artery bypass grafting. Both had multiple NSSCL. Although the proband had tendon xanthomata, none of her children or siblings had xanthomas, xanthelasmas or arcus senilis. However, no information was available on the proband's sister.

The two sons of patient No. 9, the proband's sibling, live abroad and we were, therefore, unable to study them. Both were reported to have NSSCL and hypercholesterolaemia. The two children of patient No. 6, another of the proband's siblings (Nos. 7 and 8) were examined and found to be normolipidaemic, without NSSCL.

Information on the remaining members of the family could only be obtained from the proband's history. The proband's father (No. 12) had multiple NSSCL and hypercholesterolaemia. He died of a cerebrovascular accident at the age of 54. His father (No. 13) had NSSCL

TABLE 1. Lipid values and the lipomata occurrence of each member of the family

No.	Name	Sex	Age	NSSCL	LDLC (mg/dl)	HDLc (mg/dl)	Chol (mg/dl)	TG (mg/dl)	Diseases	Died	Relationship to proband
1	A.G.	F	55	++	312	73	412	134	-	-	proband
2	A.S.	M	33	++	240	52	333	205	-	-	son
3	A.R.	F	22	+	173	64	285	235	-	-	daughter
4	A.V.	F	28	-	131	50	201	100	-	-	daughter
5	A.A.	M	59	-	166	48	257	265	MI	-	husband
6	W.Z.	M	54	++	213	51	319	274	MI	+	sibling
7	W.S.	M	25	-	91	52	161	98	-	-	son of No. 6
8	W.T.	F	12	-	86	62	160	60	-	-	daughter of No. 6
9	W.N.	M	59	++	224	48	305	164	MI	-	sibling
10	W.S.	M	35	++			high			-	son of No. 9
11	W.Z.	M	32	++			high			-	son of No. 9
12	W.Sc.	M	54	++			high		HT, CVA	+	father
13	W.M.	M	50	++					MI (?)	+	grandfather
14	W.A.	M	18						killed at war	+	sibling of No. 12
15	W.C.	M	52	++			high		HT, CVA	+	sibling of No. 12
16	W.G.	M	66						MI	+	sibling of No. 12
17	S.L.	F	60	++			high		HT, CVA	+	sibling of No. 12
18	G.D.	F	38	++			high			-	daughter of No. 12

MI = myocardial infarction; HT = hypertension.

CVA = cerebrovascular accident.

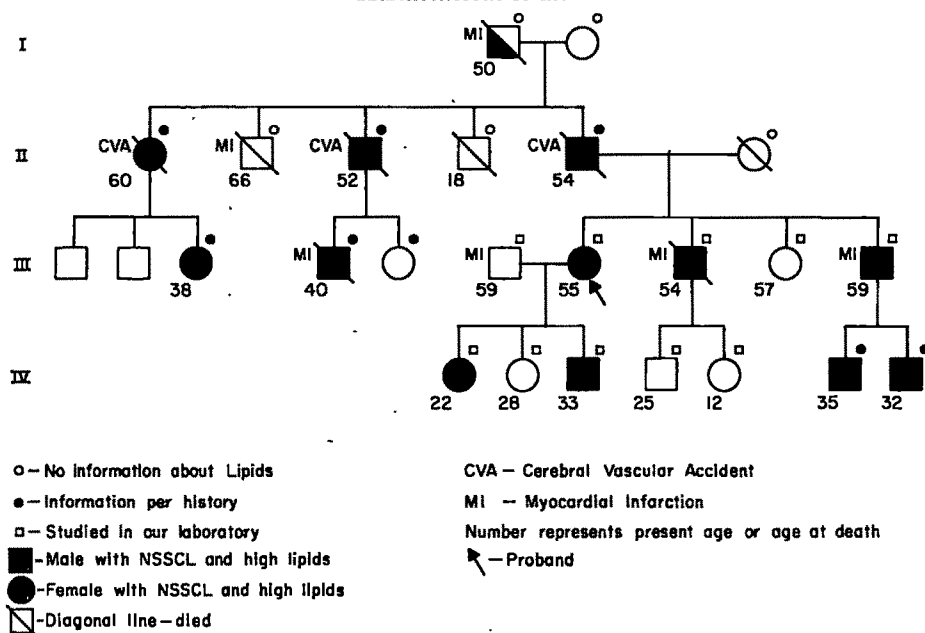


FIGURE 1. This illustrates the family tree with the ages, occurrence of NSSCL and cause of death.

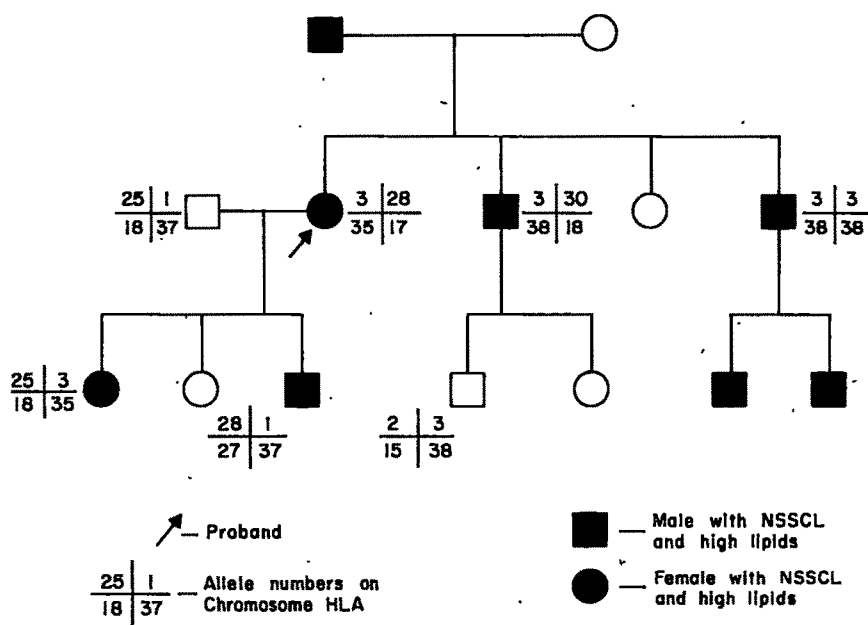


FIGURE 2. This illustrates the segregation of HLA haplotypes in the family.

and died of a myocardial infarction at the age of 50. No information was available on his serum lipid values.

Two siblings of the proband's father had hypercholesterolaemia and NSSCL. Both died of a cerebrovascular accident at the ages of 51 and 60 (Nos. 15 and 16, respectively). No information is available on the other two siblings of the proband's father or his family, except for one daughter of his two brothers who was examined in another medical center at the age of 38 and found to have hypercholesterolaemia and multiple NSSCL.

The family genealogy, with ages and causes of death, is illustrated in Figure 1.

Those members whose HLA antigen phenotype was determined are illustrated in Figure 2. The proband and her brothers did not share any HLA haplotype, nor did the proband's two sick children, indicating that association with HLA chromosomes seems unlikely.

DISCUSSION

Non-symmetric subcutaneous lipomatosis is a benign entity and normally has no clinical significance except for the cosmetic disability. It is not the result of any metabolic disturbance, and is not associated with abnormalities of lipid metabolism. It has a familial occurrence and the mode of inheritance suggests an autosomal dominant trait.¹

By contrast, multiple symmetric lipomatosis is characterized by large symmetric lipomas, mainly in the neck and shoulder region, and may be associated with hypertriglyceridaemia, elevated HDL cholesterol, hyperuricaemia, impaired glucose tolerance and renal tubular acidosis. The lipomatous tissue in these patients has been shown to have enhanced lipoprotein lipase activity,⁸ a possible explanation for the elevated HDL cholesterol levels displayed by these patients. The inheritance is also autosomal dominant.⁷

The family we describe has members afflicted with familial combined hyperlipidaemia, associated with subcutaneous lipomatosis. It is noteworthy that the proband's young daughter (No. 3) has only a few lipomas and only mild elevation of the cholesterol level. This correlation between the degree of hyperlipidaemia, and the amount of subcutaneous lipomas, possibly suggests a metabolic link between these two phenomena.

In this family, the parallel occurrence of NSSCL with an elevated level of total serum cholesterol and LDL cholesterol, as opposed to normo-lipidaemia in the subjects without NSSCL, tends to suggest that both abnormalities share a common inheritance pattern. This pattern is probably not HLA-related, as reflected by HLA studies of the family (Fig. 2).

The question of a possible metabolic relationship between the NSSCL and the hyperlipidaemia in this family is interesting. The elevated HDL levels in subjects with lipomatosis may suggest an analogy to MSL. However, we were not able to show any other feature of MSL in the examined subjects.

In a systematic review of the medical literature, we did not find a description of NSSCL combined with hyperlipidaemia. Moreover, the familial occurrence of this disease has also not been previously reported.

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Cutaneous chylous reflux

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SUMMARY

Two cases of cutaneous chylous reflux are described. Both patients had many translucent white vesicles from which milk-like fluid wept intermittently. Lymphangiogram revealed dilated, tortuous lymphatic vessels and dermal backflow. Electron microscopic studies showed an abnormal ultrastructure of the dilated lymphatics.

The cutaneous lesions associated with reflux of chyle and lymphatic fluid have received little attention in the dermatological literature. Chyle originates in the bowel lacteals and passes through the cisterna chyli and the thoracic duct and finally enters the bloodstream. The term chylous reflux refers to the backflow of chyle from its normal route. In 1964, Kinmonth and Taylor¹ reported 19 cases of chylous reflux that were divided into two types. Type I was associated with megalymphatics and type II with lymphatic deficiency. Type I patients usually suffered from lymphoedema of a lower limb with large incompetent lymphatics, chylous fistulae and a discharge of milky fluid from the lower limb and or the genitalia. A concomitant capillary haemangioma on the skin was often present. The prognosis of type I was good. Type II patients were associated with congenital or early-onset limb lymphoedema and had a prominent chylous effusion. Lymphangiograms showed subcutaneous lymph vessels that were either few or absent. Hypoproteinaemia was noted and the long-term prognosis was poor. Primary chylous reflux suggests the absence of an apparent aetiology,² and may be due to malformations of the mesenteric lymphatics.³ On the other hand, irradiation, neoplasm, trauma and infection with filariasis² can be the underlying cause in secondary chylous reflux. We report two cases of primary cutaneous chylous reflux and discuss the abnormal findings on electron microscopy of the skin.

CASE REPORTS

Case 1

A 22-year-old female suffered intermittently from weeping of creamy fluid from the anterior part of her left upper thigh over the previous 5 years. Each episode lasted for 4 to 5 days. Her

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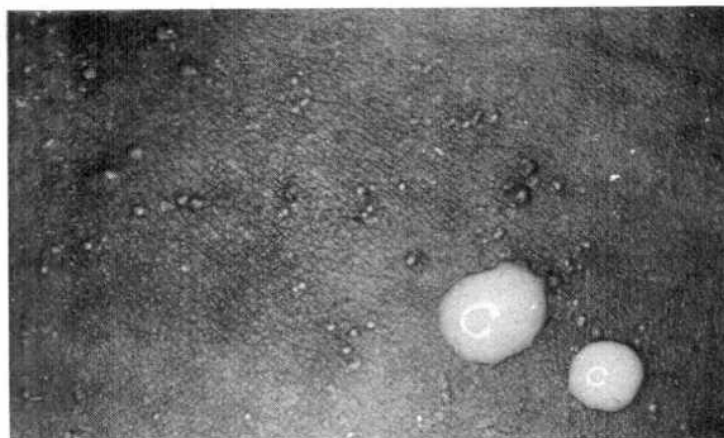


FIGURE 1. Multiple pin-head sized, firm, whitish papules which drained milk-like fluid on anterior femoral region of left thigh (Case 1).

growth, development and general health remained normal and no other family members had a similar skin disorder.

Examination showed numerous small, firm, whitish vesicles on the anterior part of her left upper thigh (Fig. 1). Milk-like fluid from these ruptured vesicles collected (Fig. 2). The patient had no associated oedema of the leg.

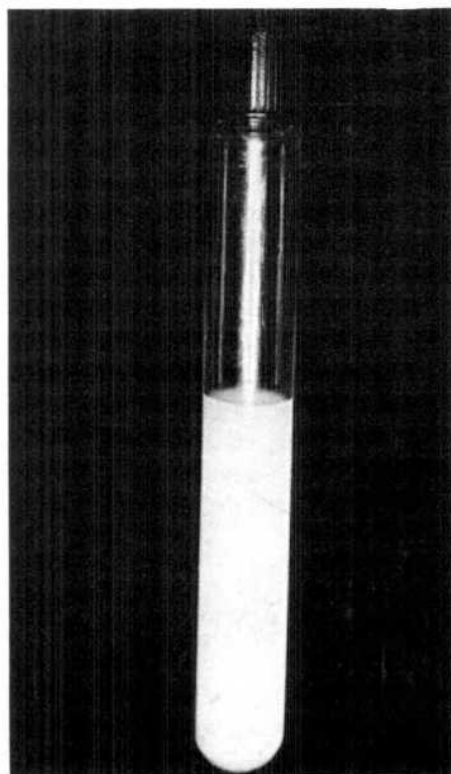


FIGURE 2. Drainage of milk-like fluid from skin lesions for 30 min (Case 1).

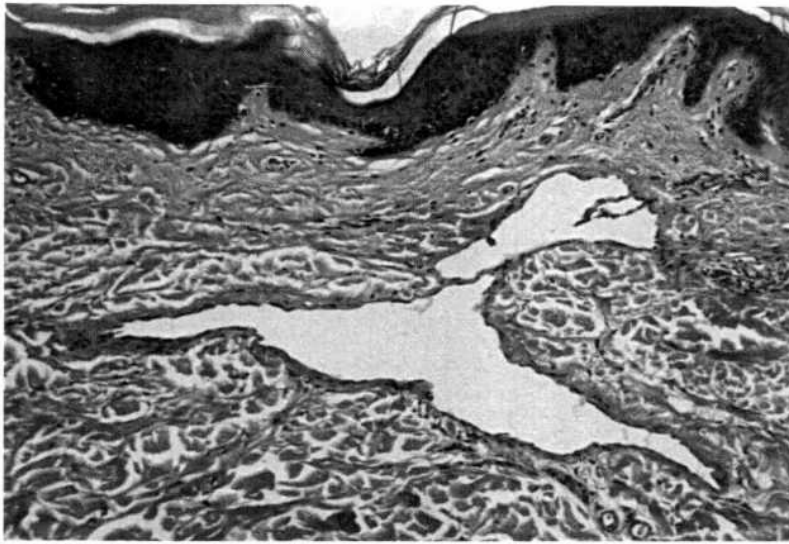


FIGURE 3. Dilated lymphatic lumens lined by a single layer of endothelial cells in dermis (Case 2). (H&E, $\times 100$).

Laboratory studies, including haematology, urinalysis, stool examination, fasting blood sugar, blood lipids, lipoproteins, blood-urea nitrogen, serum creatinine, electrolytes, serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were all normal. The cholesterol and triglyceride in the chyle were 520 mg/dl and 1750 mg/dl respectively. A lymphangiogram showed dilatation and tortuosity of the left iliac lymphatics and a dermal backflow on the upper medial part of the left thigh. An abdominal CT scan was normal and did not show any tumours.

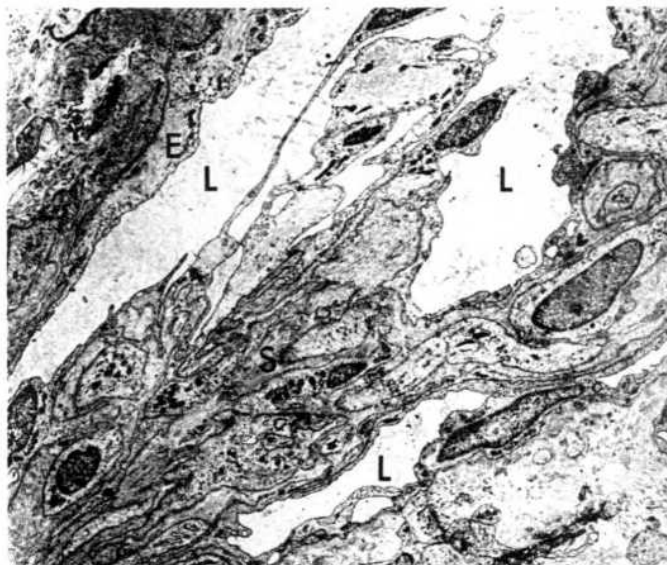


FIGURE 4. Ultrastructure of intraluminal septum of lymphatic vessels. S: septum, E: endothelial cell, L: lymphatic lumen (Case 1). ($\times 4400$).

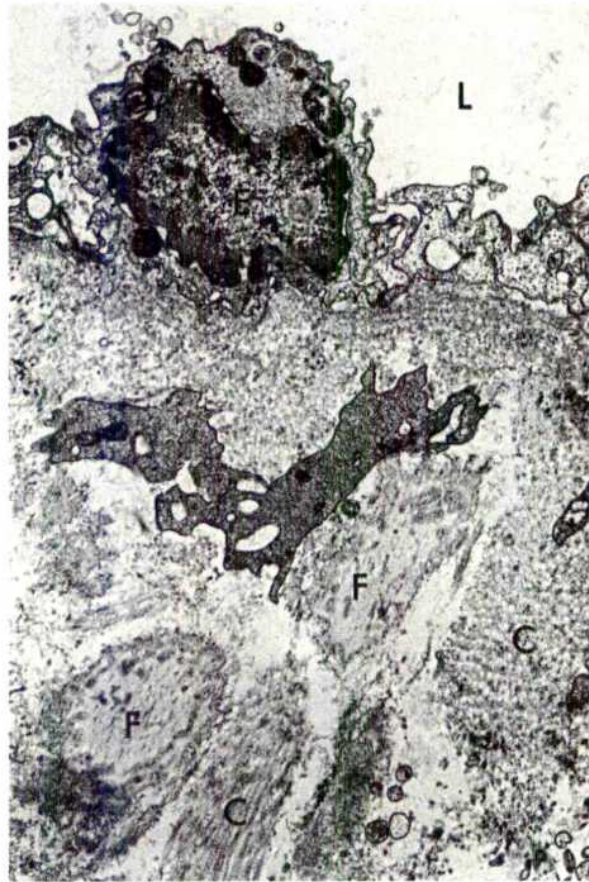


FIGURE 5. Elastic fibres located adjacent to the endothelial cell (Case 2). L: lumen, E: endothelial cell, C: collagen fibres, F: elastic fibres. ($\times 18000$).

Case 2

A 57-year-old male had two episodes in which there was weeping of creamy fluid in the left pubic region in the previous 10 months. Each episode cleared spontaneously with bed rest in 1 week. No other members of his family were affected.

Examination showed no other abnormality apart from the skin problem. The pubic hairs were matted with yellowish crusts and there were numerous pin-head sized, firm, white vesicles that were located on left side of the pubic region. Milk-like fluid drained from these ruptured papules continuously.

Laboratory studies were all normal. A lymphangiogram showed immediate filling with dilatation and tortuosity of the superficial lymphatics of the left thigh and the presence of collateral lymphatics in the pelvis. There was no evidence of a tumour mass or lymph node enlargement on CT scans of the abdomen and pelvis.

HISTOLOGY

Histological examination of skin biopsies from cases 1 and 2, showed dilated lymphatics with valves in the upper dermis that were lined by a single layer of endothelial cells and with only few surrounding elastic fibres (Fig. 3). Ultrastructurally these lymphatics in the upper dermis were dilated and their walls consisted of a single layer of endothelial cells, that connected by open and loose junctions, with overlapping cell processes and interdigitations (Fig. 4). The endothelial cells were of various shapes and sizes. Free spaces between the endothelial cells and the adjacent collagen fibres were found. The endothelium was crumpled and produced irregular folding of the luminal surface. There were mainly two types of intralymphatic valve. One was an individual cell that protruded into the lymphatic cavity acting as a valve. The other consisted of folds of the endothelium with a core of connective tissue and mesenchymal cells extending into the lumen. The surrounding basement membrane was either interrupted or absent. Collagen fibres and elastic fibres were seen adjacent to the endothelial cells (Fig. 5). Pericytes and smooth muscle cells were found occasionally and were around the large dilated lymphatics.

DISCUSSION

It has been considered that malformation of the cisterna chyli and mesenteric lymph node abnormalities were the two basic anatomic lesions involved in primary cutaneous chylous reflux.³ Clinical manifestations of cutaneous chylous reflux have been noted to vary according to the velocity of the chylous reflux.⁴ Slowly forming white solid lesions were local accumulations of whitish or creamy chyle that were usually mixed with macrophages and histiocytes. Vesicles or blisters, however, were formed under more rapid and forceful chylous reflux. These vesicles are not true ones but dilated cutaneous lymphatics which approach the epidermal surface and mimic vesicles clinically. The chyle may discharge from these lesions either constantly or intermittently. The skin lesions usually appear on the external genitalia, thighs,¹⁻⁴ the abdominal wall, feet and knee joints.³ Lymphoedema of a limb is common finding and may occur before or after the skin eruption. Our two cases had only cutaneous lesions and so far no lymphoedema of a limb.

Electron microscopy of the skin biopsies revealed little ultrastructural abnormality of the lymphatics, though free spaces were apparent between the endothelial cells and the adjacent connective tissue. The precise nature of this finding is unclear. Another finding was the presence of the irregular thickened intralymphatic folds that acted as valve-like structures and were not seen in normal lymphatics.

There is no treatment for this skin disorder though ligation and excision of the incompetent lymphatics above the area of chylorrhea has been tried but recurrence of the disorder occurred. Total superficial lymphangiectomy on patients of cutaneous chylous reflux having lymphoedema has also been carried out.³ Our cases did not have lymphoedema, and they only received conservative treatment.

ACKNOWLEDGMENTS

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Imported rickettsial African spotted fever in Japan

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SUMMARY

A 45-year-old Japanese man developed a black eschar skin lesion and on the body, a number of erythematous seropapules 2 weeks after his return from South Africa. Within 24 h of treatment with minocycline he was afebrile and symptomatically much improved. Serological tests showed increased antibody titres to spotted fever group rickettsiae (SFGR). From the clinical and serological findings we consider this patient to be the first in Japan to have been infected with SFGR in Africa.

International travel is common and modern transportation results in a situation where persons harbouring infections acquired in regions where such diseases are endemic can return to countries where such diseases are rare or absent. We report on a patient from Yokohama whom we consider to be the first Japanese case of South African spotted fever.

CASE REPORT

A 45-year-old Japanese man was referred to our clinic on February 24, 1988. He had visited Kimberley, South Africa, early in February 1988. During his stay, he went into the bush and was unaware that he had received a tick bite on his left buttock. Two weeks after returning to Japan he developed tender, enlarged lymph nodes in the left inguinal region. He then developed malaise associated with headache and fever (39°C). An extensive indurated and erythematous papular rash appeared, and covered the whole body (Fig. 1). A non-tender, 4-mm lesion with a black eschar surrounded by an area of erythema, was present on the left buttock (Fig. 2). Laboratory studies disclosed the following values: haemoglobin, 14.5 g/dl; platelets 182,000/ml; leukocyte count, 2,800/ml with 1% metamyeloblasts, 1% band cells, 29% segmented neutrophils, 13% mononuclear cells, 55% lymphocytes and 1% atypical lymphocytes. A skin biopsy specimen of the lesion on the buttock showed necrotizing vasculitis with a perivascular infiltrate consisting mostly of lymphocytes and macrophages of the dermis and subcutaneous tissue (Fig. 3). After the skin biopsy, the patient was given 200 mg oral minocycline hydrochloride daily, for 5 weeks. Within 24 h he was afebrile and symptomatically improved.

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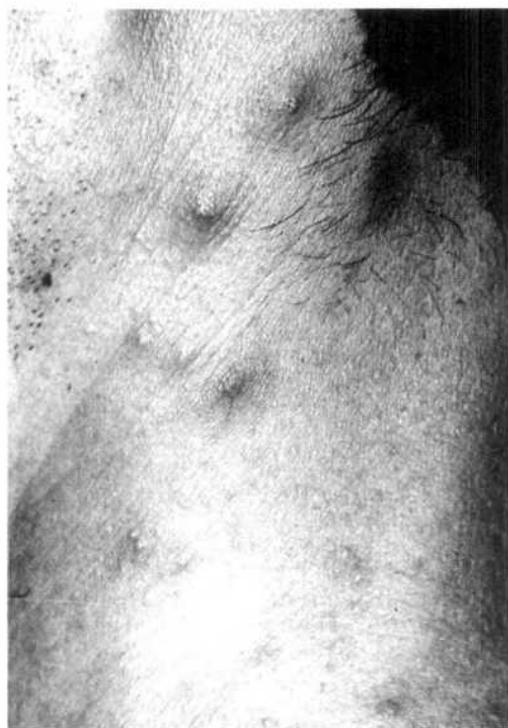


FIGURE 1. Indurated erythematous papular rash on the left neck.

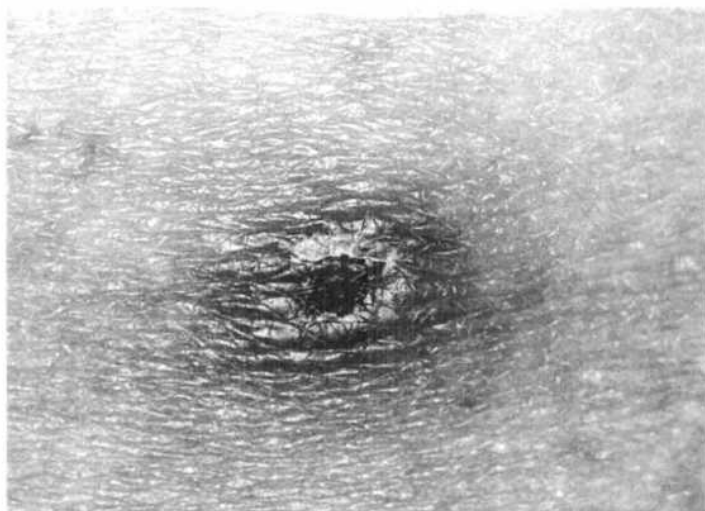


FIGURE 2. A non-tender 4 mm lesion with black escher on the left buttock.

FIGURE 3. Necrotizing vasculitis in the eschar. (H&E stain, $\times 40$.)

Within 72 h the rash had almost disappeared and the lymphadenopathy had decreased. Indirect immunoperoxidase (IP) tests¹ were carried out and the results of these serological tests are shown in Table 1. These showed a clear elevation of both IgG and IgM antibodies against SFGR, but *Rickettsia tsutsugamushi*, and the Weil-Felix reaction was negative. These results indicated that the patient had been infected with a tick-borne typhus rickettsia, possible *R. akari* or *R. conori*, in South Africa. However, it was impossible to further discriminate between the two rickettsiae.

TABLE 1. Antibody titres

Date	<i>R. tsutsugamushi</i>						SFGR				Weil-Felix		
	Gilliam		Karp		Kato		TTT		Katayama				
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	OXK	OX2	OX19
February 24, 1988	<10	<10	<10	<10	<10	<10	<10	<10	≤10	<10	<10	<10	<10
March 2, 1988	<10	<10	<10	<10	<10	<10	640	≤10	1280	40	<10	<10	<10
March 14, 1988	<10	<10	<10	<10	<10	<10	2560	80	2560	80	<10	<10	<10

DISCUSSION

The SFGR infection is a tick-borne disease that is caused by *R. akari* and is endemic in Africa, North America, the Soviet Union and Korea. The incubation period is about 7 days and the patient becomes pyrexial about 10 days after infection. The lesion starts as a small area of erythema and soon develops into a vesicle which later becomes an eschar with an associated enlargement of the regional lymph glands. Malaise and headache are invariably present. Widespread maculopapular eruptions appear and these cases are often diagnosed as atypical varicella. However, serological tests confirm the diagnosis of SFGR.

The SFGR infection, boutonneuse fever, a tick-borne typhus caused by *R. conorii*, occurs throughout the African continent, in European and Middle Eastern areas adjacent to the Mediterranean, Black and Caspian Seas, and in India. Boutonneuse fever in tourists has been reported in the Netherlands, Germany, Switzerland² and the U.S.A.³

Boutonneuse fever is a mild to moderately severe illness with an incubation period of 5-7 days. In a typical case, the patient has a fever lasting several days to 2 weeks with an initial lesion, an eschar or 'tache noire', and a more generalized erythematous maculopapular rash that appears on about the fifth day and often involves the palms and soles.⁴ The eschar which occurs at the site of tick bite is typically seen on the covered parts of the body and resembles a cigarette burn. It is often accompanied by regional lymphadenopathy. The diagnosis is confirmed by seroconversion to *R. conorii* antigen, or to the closely related antigen of SFGR. Our patient had travelled to South Africa, where SFGR, a tick-borne typhus due to *R. akai* and *R. conorii*, is an endemic disease. On his return to Japan he complained of lymphadenopathy and headache and an eschar was present on the left buttock, and minocycline proved very effective. He had never been to other areas endemic for SFGR apart from South Africa.

Serological tests for SFGR were clearly positive for both IgG and IgM and indicated that the patient had been infected with SFGR. The TT-118 (TTT) strain used in this study had been isolated from ticks in Thailand in 1962 by Elisberg and his co-workers.⁵

On the basis of the clinical course, with the development of indurated erythematous seropapules resembling atypical varicella, it seems likely that our patient was infected in Africa by a tick carrying *R. akari* rather than *R. conorii*.

Since long distance travel is now very common, it is important to be aware of and recognize rickettsial infections and carry out the appropriate serological tests.

ACKNOWLEDGMENT

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Prurigo pigmentosa successfully treated with minocycline

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SUMMARY

Five patients with prurigo pigmentosa were treated with 100–200 mg minocycline daily. The eruption and pruritus rapidly resolved within a few days or up to a week. There has been no recurrence after stopping medication and we conclude that minocycline is effective for prurigo pigmentosa and safer than dapsone.

Prurigo pigmentosa was first described in 1971 by Nagashima *et al.*,¹ as an inflammatory disorder characterized by pruritic red coloured papules with gross reticular pigmentation on the chest, back or neck. It affects mainly Japanese people and particularly young females, although non-Japanese cases have been reported.^{2–4} Dapsone and sulphamethoxazole are effective treatments for this condition. We describe five cases of prurigo pigmentosa that were successfully treated with minocycline.

CASE REPORTS

Case 1

A 16-year-old boy developed pruritic red papules with erythema on his upper back and lumbar region in January 1987. The eruption spontaneously disappeared to leave a reticulated brown hyperpigmentation. However, the eruption frequently recurred and minocycline therapy was started in August. Within a few days the pruritus and rash had reduced through minocycline, given 200 mg daily for 2 weeks, and the 100 mg daily for a further 4 weeks and then stopped. Over the past 12 months there has been no recurrence.

Case 2

A 17-year-old girl had pruritic papules and a reticular erythema with pigmentation on the nape of her neck in May 1987. Treatment was with minocycline 200 mg daily, and within 1 week the

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pruritic papules and erythema developed into a reticulated pigmentation of the skin. Minocycline was continued in a dosage of 50–100 mg daily for a further 6 weeks and then stopped. No recurrence has been observed over the past 14 months.

Case 3

A 16-year-old girl with diabetes mellitus had pruritic papules and erythema on her neck and upper back in April 1988. Minocycline was given in a dosage of 100 mg daily for 3 weeks and within the week the eruption had disappeared to leave slight residual hyperpigmentation. No recurrence of the rash has occurred in the past 4 months.

Case 4

A 15-year-old girl developed pruritic papules and erythema in the scapular region in March 1986. After 2 months, the eruption spontaneously disappeared, leaving residual pigmentation. It recurred many times without any provocation and then spread to involve the nape of the neck, chest and back. In September 1987, dapsone 50 mg daily was given but though effective, the condition relapsed on stopping the drug. In May 1988, minocycline 200 mg daily was started and, after a few days, the pruritus and rash cleared but left a marked reticulated hyperpigmentation (Fig. 1). Minocycline 100 mg daily was continued for a further 3 weeks and then stopped. No recurrence has been observed over the past 4 months.

Case 5

A woman first noticed pruritic red papules with erythema on her chest, back and neck in 1973 when aged 19. Treatment with dapsone 25 mg daily or sulphamethoxazole 1–2 g daily was given from 1977 to 1978 and both drugs were effective, but the rash recurred on stopping the drugs. She discontinued therapy because of marriage and pregnancy for 8 years though eruptions recurred about once a month without any specific provoking factor. In May 1988, minocycline 100 mg daily was started and further recurrences were prevented. Since she stopped the drug there has been no recurrence.



FIGURE 1. Red papules and erythema rapidly disappeared leaving gross reticulated hyperpigmentation after minocycline therapy (Case 4).

DISCUSSION

Minocycline therapy of prurigo pigmentosa was first reported in 1979 by Tashiro,⁵ who successfully treated two patients. Minocycline therapy for this condition is not well known even in Japan, because often dapsone is used as the first choice in treating prurigo pigmentosa. We evaluated the effect of minocycline on prurigo pigmentosa and in all our cases it was most effective. With a dose of minocycline 100–200 mg daily, the pruritus, papules and erythema rapidly disappeared within a few days or up to a week. Although prurigo pigmentosa is likely to recur and can continue for many years, minocycline is effective in preventing recurrence and in two of our cases where the eruption recurred after stopping dapsone therapy, a long remission was obtained with minocycline.

The action of minocycline on prurigo pigmentosa is not clear and the aetiology of prurigo pigmentosa is likewise unknown. Minocycline is a semi-synthetic tetracycline known to have anti-inflammatory properties and tetracyclines inhibit neutrophil chemotaxis^{6,7} and mitogenic response of lymphocytes.^{8,9} Tetracyclines including minocycline have been reported to be effective in various inflammatory skin disorders cystic acne, pyoderma gangrenosum,^{10,11} bullous pemphigoid,^{12,13} alopecia mucinosa,¹⁴ and eosinophilic pustular dermatosis.¹⁵ These disorders are also responsive to dapsone. Minocycline may have similar anti-inflammatory effects to dapsone; i.e. an inhibition of oxygen intermediates in inflammation.^{16,17}

Although dapsone and sulphamethoxazole are effective for prurigo pigmentosa, these produce severe side-effects such as methemoglobinemia, agranulocytosis, haemolysis and hepatotoxicity.¹⁸ Minocycline has been widely used in the treatment of acne vulgaris and has been found to be not only effective but safe. We conclude that minocycline is of help in the treatment of prurigo pigmentosa and safer than dapsone and sulphamethoxazole.

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Regression of oral Kaposi's sarcoma in a case of AIDS on Zidovudine (AZT)

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SUMMARY

A case with oral Kaposi's sarcoma (KS) is reported that regressed during therapy with Zidovudine (AZT) which was started 5 months after the first dermal and oral tumours were noted. After 6 months of treatment the absolute number of T-helper cells had increased from $54/\mu\text{l}$ to $232/\mu\text{l}$ and the ratio of T-helper to T-suppressor cells from 0.15 to 0.3. During the same time the lesions of KS on the gingiva, uvula and the body as well as the face disappeared. The lesion of KS on the hard palate regressed.

Kaposi's sarcoma (KS) associated with AIDS was first noted in homosexual men in 1981.¹ Since then the incidence of KS in AIDS has decreased from 30%² to 15%³ of AIDS cases. In most patients the KS tumours occur intraorally, often in the area supplied by the palatine vessels.⁴

Clinical studies with the antiviral drug AZT were started in the U.S.A. in July 1985. Patients on AZT showed a marked increase in T₄ counts, development of delayed hypersensitivity and weight gain.^{5,6} Regression of one or more nodules of AIDS-related KS has been observed in some patients, and regression of all AIDS-related KS lesions has been reported in six patients.

The purpose of this report is to describe the clinical, histological and immunological findings in a patient who developed regression of AIDS-related oral KS during therapy with AZT.

CASE REPORT

A 27-year-old male homosexual patient was found, in May 1987, to be HIV₁ positive. At that time, a number of pigmented macules on the body were excised and the histology was that of KS. The ratio of T-helper (T₄)/T-suppressor (T₈) lymphocytes was 0.61 and the absolute number of T-helper cells was $320/\mu\text{l}$.

In June the patient had rapidly growing oral lesions in relation to the upper and lower incisors (Fig. 1a) and also in the middle of the hard palate. The absolute number of T-helper cells was $260/\mu\text{l}$, the ratio of T_4/T_8 was 0.46. Histological examination of the gingival lesion revealed vessel-like spaces with extravasated erythrocytes, spindle-shaped cells, and a slight inflammatory infiltrate and confirmed the clinical diagnosis of oral KS (Fig. 2a). New and rapidly growing lesions were seen on the uvula in the next 3 weeks. By 7 September the patient complained of weight loss and general fatigue. The T_4/T_8 ratio was now 0.15 and the absolute number of

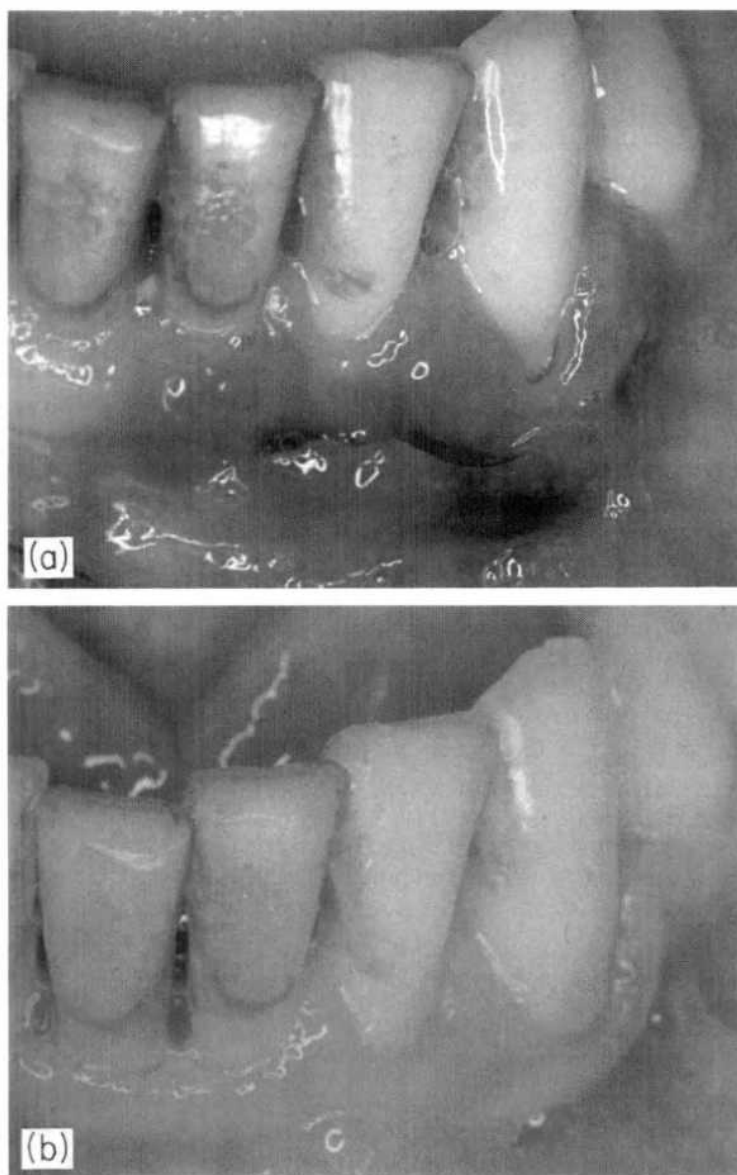


FIGURE 1. (a) Rapidly growing KS in the area of the left lower incisors (September 1987). (b) After 6 months the lesions were clinically dissolved, leaving loss of interdental papillae and recession of gingival margins (February 1988)

T-helper cells was $54/\mu\text{l}$. Tests for *Toxoplasma gondii*, *Candida albicans* species, *Cryptococcus neoformans* and atypical mycobacteria were negative. Culture of the urine showed cytomegalovirus (CMV) and the patient's serum contained IgG antibodies to CMV in titres of 1:1024, but not anti-CMV IgM antibodies. The Merieux (multi-allergen) test was negative. Computerized cerebral tomography revealed no abnormality. Treatment with AZT in a dosage of 200 mg six times a day was started on 9 September, and by 22 September, the oral KS lesions in the area of the upper incisors seemed smaller in size.

At the end of October skin lesions of the KS were softer in texture and the previously rapid tumour growth had stopped. The body weight had increased to 54.3 kg. The ratio of T_4/T_8 cells was 0.4 and the absolute number of T-helper cells was $250/\mu\text{l}$. The number of leucocytes was decreased ($2.9 \times 10^9/\text{l}$), and the haemoglobin level was 11.4 g/dl. The Merieux skin test

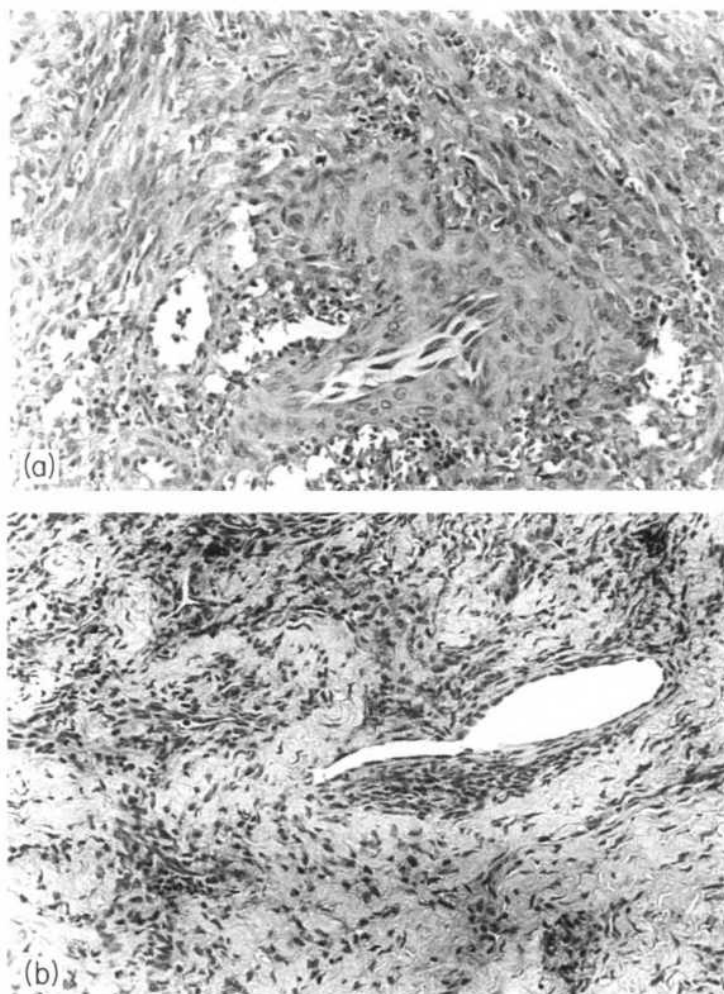


FIGURE 2. (a) Histological section of the KS lesion seen in Figure 1(a) showing characteristic slit-like channels, extravasated erythrocytes and hemosiderin and spindle-shaped cells (H&E $\times 175$) (b) Histological section of the gingiva of the lower incisors seen in Figure 1(b). The vascular spaces are lined by regularly shaped endothelial cells; a moderate inflammatory infiltration of the dense collagenous tissue is seen (H&E $\times 175$)

remained negative. On follow-up, in February 1988, the oral KS in the area of the upper and lower incisors (Fig. 1b), on the uvula and in the area distally to the right lower molar had disappeared. A further biopsy of this area revealed only mild inflammatory cell infiltrate with some vascular spaces and regularly shaped endothelial cells (Fig. 2b). The lesion of KS involving the hard palate was reduced in size and the mucosa showed a more normal colour. Apart from haemosiderin pigmentation of scars on the body and on the face, following the excision of KS lesions, there were no signs of the previous lesions. The Merieux test was still negative. The absolute number of T-helper cells was $232/\mu\text{l}$, and the ratio of T-helper/T-suppressor cells was 0.3. The number of leucocytes $2.2 \times 10^9/\text{l}$ and the haemoglobin level 9.2 g/dl. The dose of AZT was reduced to 100 mg six times a day.

DISCUSSION

KS is the most common malignant tumour associated with AIDS.^{7,8} Many patients have a fulminant course of the disease with early dissemination and multiple organ involvement with KS.⁹ Patients with only few slow-growing lesions have usually a normal or only a moderately impaired immune system and severe immune impairment may not be prerequisite for the development of KS lesions.⁸ Some patients may have spontaneous regression of one or more nodules of KS,¹⁰ but spontaneous regression of all AIDS-related KS lesions has only been reported in six cases. Janier *et al.*¹¹ reported one case of AIDS-related KS, with spontaneous regression of the lesions, though the patient deteriorated and died within 2 months of opportunistic infections. Real *et al.*¹² described five AIDS patients with spontaneous regression of KS and when regression occurred, they had only a few lesions and none had a history of opportunistic infections. The clinical course of our case is different from that usually seen. The lesions occurred in a patient with severe immunological impairment and the lesions rapidly developed and were disseminated. This indicated a rapid and progressive course for the disease. During therapy with AZT the number of T-helper cells increased slightly and remained at the same level during the subsequent 6 months.

Placebo-controlled studies have shown that AZT decreases mortality and the frequency of opportunistic infections in patients with AIDS or AIDS-related complex (ARC).⁵ Patients on AZT had a significant improvement of immune function. In a study of 284 patients with AIDS or ARC, KS lesions developed in 16.⁵ Six were patients on treatment with AZT ($n = 145$; 4.1%) and ten were in the placebo group ($n = 137$; 7.3%).⁵

Our patient did not receive any other therapy and we suggest that the regression of KS was due to AZT, however it is possible that this regression was spontaneous. Further studies are needed to evaluate the effect of AZT in AIDS-related Kaposi sarcoma.

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Addendum in proof

16.2.89: the patient is still on medication with AZT (4×250 mg/day), is clinically well and his KS remained in regression.

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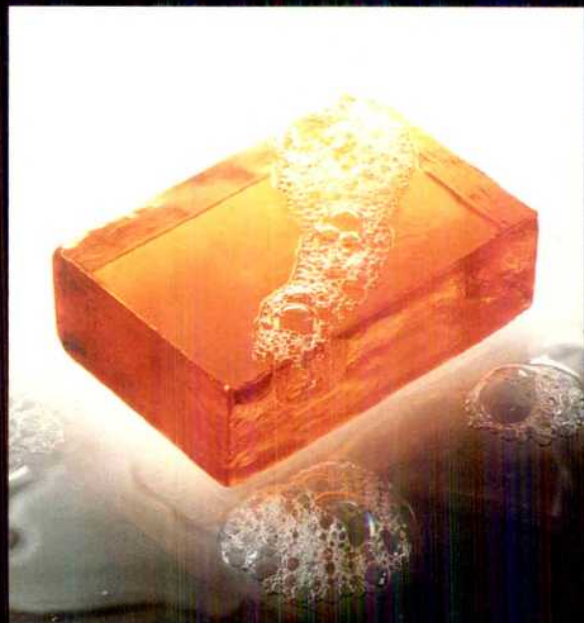
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Premenstrual exacerbation of atopic dermatitis

SIR, We have recently been impressed by the observation, in several young women with atopic dermatitis, of a cyclical variation in the severity of their disease, particularly in the week prior to menstruation. In order to investigate this phenomenon further, a questionnaire was designed and sent to 204 women (aged 15–45 years) with atopic dermatitis. This represented an unselected sample of patients attending for outpatient treatment. Replies were received from 143 (response rate 70.1%).

They were asked to state, considering their previous menstrual cycles, the times when their skin condition was at its best and worst. There were five possible time responses, namely 2 weeks and 1 week prior to menstruation, the week of menstruation and 1 and 2 weeks after menstruation. Of the 143 replies, 41 women (28.7%) replied that their skin was worse in the week before menstruation with improvement in the week during or after menstruation. Another 13 (9.1%) patients stated that their eczema was worse at other times. The remaining women did not notice a consistent variation with their menstrual cycles.

We are not aware of any previous reports of premenstrual exacerbation of atopic dermatitis. However, it is of interest that atopic asthma can be exacerbated premenstrually, as reflected by a modest fall in peak expiratory flow rate, and this is recognized in approximately one-third of affected women.^{1,2} It appears unrelated to symptoms of the premenstrual tension syndrome or to the use of oral contraceptives. This phenomenon of a premenstrual flare of atopic dermatitis focuses attention on the possible influence of endocrinological factors in the expression of dermatitis reactions.

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Purpura and sepsis—rarer causes?

SIR, The list of infective causes of purpura is exhaustive and well documented.^{1,2} However, there are puzzling and often fatal septicaemic illnesses caused by the fastidious Gram-negative organism, *Dysgonic Fermenter* type-2 (DF-2),^{3,4} that sometimes go undiagnosed. Of the 52 cases of DF-2 infections reported worldwide,⁵ 26 (50%) had dermatological manifestations which included: petechiae/purpura (11), macular/papular erythematous lesions (9), symmetrical peripheral gangrene (3), eschars (2) and painful erythema (1). Thirteen out of the 52 cases had cellulitis on presentation. DF-2 appears to be an opportunistic pathogen of low virulence⁶ and all age groups are vulnerable. Although there are well recognized predisposing factors (a third of the patients had splenectomies, a quarter were alcoholics and 15% had chronic respiratory illness), a fifth of all patients were previously healthy.⁵ Infection with DF-2 carries a high mortality and 13 out of the 52 patients died.

Many physicians are unaware of the existence of this zoonosis associated with canine contact, especially dogs and cats. The clinical and pathological features of this infection are indistinguishable from septicaemia due to other bacteria,⁵ and isolation of the organism is difficult. A history of animal contact in a seriously ill patient with skin lesions and the established predispositions must alert the physician to the possibility of infection with DF-2. Empirical intravenous antibiotic treatment must be instituted on clinical suspicion alone. DF-2 is sensitive to most antibiotics except aminoglycosides (Penicillin G is the

treatment of choice). Purpura and maculo-papular lesions have several potentially fatal underlying causes and are extremely important and sinister physical signs.

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Concomitant Becker's naevus and acanthosis nigricans

SIR, Buck, Tatnall and Sarkany (*British Journal of Dermatology* 1988; **118**, Suppl. 33: 99-100) reported a female patient with acanthosis nigricans, insulin resistance, hyperandrogenism and unilateral breast hypoplasia. The photograph published with this report is remarkable in two respects. Firstly, acanthosis nigricans (AN) is usually symmetrically distributed and may be localized in the neck, axillae, sub-mammary folds, umbilicus, groins, anogenital area, antecubital and popliteal fossae. The persistent unilateral localization of AN completely covering a single breast in this case is curious. Secondly, the pigmentation of the large macule and the many hyperpigmented islands at its peripheral border covering the hypoplastic breast and subaxillary area appears to be more intense than the pigmentation in the left axilla. This suggests the concurrence of two separate anomalies that share the clinical feature of hyperpigmentation.

We wonder whether the authors have considered the possibility in their case of a concomitant melanosis naeviformis of Becker (MNB) and AN. MNB usually consists of a unilateral localized hyperpigmented macular lesion in which hypertrichosis may later develop. The shoulder scapular region and upper chest are the usual sites of MNB. This may be congenital, but the peak incidence of onset is at puberty and in early adolescence. Various developmental defects of soft tissue and bone may be associated with MNB.¹ Ipsilateral breast hypoplasia with, in most instances, overlying MNB has been reported in five female patients.¹⁻⁵

Histological examination MNB and AN may be easily confused because both may share main features of orthohyperkeratosis, acanthosis, papillomatosis and hyperpigmentation of the basal layer.

The concurrence of MNB, AN and acne vulgaris has been described.⁶ Furthermore we have observed an obese 30-year-old man with a non-hypertrichotic MNB localized to the right side of the chest and shoulder and with AN in the neck, axillae and sub-mammary region, multiple skin tags in the neck and the right axilla and truncal acne vulgaris. All the anomalies in this patient developed at the age of 18. Insulin resistance and serum androgen levels were not determined in this patient. The coexistence of MNB and AN (and even acne vulgaris) may not be fortuitous since it has been suggested that the development of all these is influenced by androgen stimulation.^{6,7} In our opinion the report of Buck *et al.* represents a

concomitant MNB associated with ipsilateral breast hypoplasia and AN associated with insulin resistance and hyperandrogenism, which makes their case more interesting.

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The use of water as a mounting medium for light microscopy to study the surface architecture of hair

SIR, During a recent study of scalp hairs of premature babies, D.P.X. was not available as a mounting medium and water was used instead. The surface architecture using water showed up more distinctly (Fig. 1) than when D.P.X. was later used to examine similar hairs from the same patient (Fig. 2). The best results were obtained when the light intensity was reduced by racking down the condenser and/or by closing the objective diaphragm. The same findings were obtained when other hairs were examined. Phase contrast microscopy did not prove as effective as water. The use of water as a mounting medium is cheap, simple, quick and informative.

I would like to thank Dr S.W.B. Ewen, Department of Pathology, Medical School, Aberdeen for helpful advice and for the photo-micrographs.

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FIGURE 1. Light microscopy of scalp hair using water as the mounting medium.

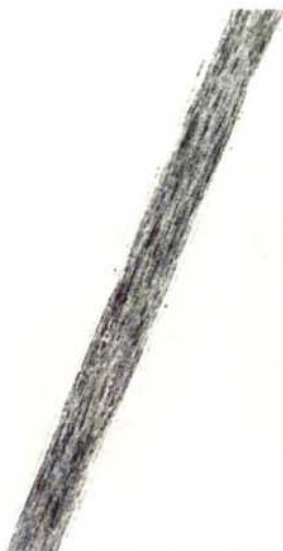


FIGURE 2. Light microscopy of scalp hair from the same patient as Figure 1 using D.P.X. as mounting medium.

Book Reviews

Diseases of the Veins, Pathology, Diagnosis and Treatment. N.L.BROWSE, K.G.BURNAND AND M.L.THOMAS. (1988) London: Edward Arnold. Pp. 694. Price £125.

At first glance this book may not seem applicable to the Dermatologist, yet much of his/her workload relates to manifestations of venous disorders, not least leg ulcers. Therefore, a full understanding of venous physiology and pathophysiology, as well as knowledge of current techniques of investigation, should be familiar to any specialist dealing with these clinical problems. For example, access to Doppler pressure measurements (ankle/brachial systolic pressure indices) is mandatory before the application of compression bandages to an ulcerated leg. *Diseases of the Veins, Pathology, Diagnosis and Treatment* serves as a reference book on all aspects of venous disease. The authors, all foremost authorities in their field, have produced a well written book which is beautifully illustrated. Chapters on 'Physiology and functional anatomy' and 'Techniques of investigation' are particularly useful. Principles of photoplethysmography, foot volumetry as well as just about every other method of assessment are explained in clear, concise terms. Even simple terms such as gold standard, sensitivity and specificity are defined, indicating the relative importance and accuracy of each test. Unfortunately, only seven lines are devoted to Duplex ultrasound/Doppler scanning, a new and important advance in dynamic imaging. This lapse probably reflects the time taken for publication of such large textbooks.

The section on venous ulceration is comprehensive, not surprisingly as Browse and Burnand are responsible for the currently upheld hypothesis on the aetiology of venous ulcers, namely the 'Fibrin cuff theory'. However, more attention could have been given to external compression (bandages and stockings). This, combined with elevation when resting, is the cornerstone of good venous ulcer management and prevention. There was no mention of the prescription changes on elastic hosiery which occurred in April 1988 and no practical information on the delivery and fitting of hosiery at the point of need, i.e. in the community. Little detail was given to care of the surrounding skin of a leg ulcer which in my experience is what district nurses find most difficult to manage. Overall a valuable addition to the library as a reference book.

P.S.MORTIMER

Textbook of Hematology. SHIRLYN B.MCKENZIE. (1988) Philadelphia: Lea & Febiger. ISBN 0-8121-1096-X Pp. 507. Price £28.00.

One of the most difficult stages in writing a book is to identify the principal readership and the range which may also be embraced if the book is to be successful. This is stated clearly in the preface and the contents remain more or less faithful to the needs of students and medical technologists.

The short historical sections are very welcome and the graphics are, for the most part, explicit and accurate but the photographs (plates i–iv excluded) are disappointing in comparison with the many other well illustrated textbooks.

There is some duplication of material—notably in the separate chapters on 'General Aspects and Classifications of Acute Leukaemia' and 'Acute Leukaemia'—which is unusual in what is predominantly a single author work but, for the most part, the text is well integrated and logical in its arrangement.

The book will probably be most useful to laboratory technologists whose needs have not been so carefully singled out for special attention and in this context should find a place in the libraries of most departments of haematology. It will probably not be of particular value to dermatologists in preference to the many other excellent textbooks of haematology which are available.

J.REES

Skin Cancer: Recognition and Management. Edited by R.S.SCHWARZ. (1988) New York: Springer-Verlag; Pp. 429, illustrations—245 black and white, 8 colour ISBN 0-387-96612-9. Price DM 198.

This is a somewhat individual book, edited by Dr. Schwarz of New Jersey. Approximately half the text is written by Dr. Schwarz himself, and the remainder by 20 collaborators. The chapters give an account of cutaneous malignant tumours, with benign lesions included in appropriate chapters, more on a differential diagnosis basis than as separate entities. There are copious references at the end of each chapter, and frequent personal impressions in the text. I personally would have found the references more useful if there had been sub-sections so that the reader could obtain some guidance as to which references were seminal articles in the field, and which were brief letters to a journal. Pathological details are given in individual chapters, and there is a comprehensive chapter on skin tumour pathology. Some cross referencing would have been of value in this section.

The black and white illustrations are of varying quality and it is disappointing in a book of this price to find only two pages of colour illustrations tucked in approximately a third of the way through the text. This book is most likely to be of interest to those committed to skin cancer studies, as the varied style of the chapters and lack of colour illustrations do not make it ideal for the newcomer. There are also some slightly doubtful treatment suggestions, such as the use of cautery for melanoma.

Although a useful source of references, the rather varied style in this book makes it less than ideal for the newcomer to skin malignancy. It will, however, be of great interest to those who are committed to the field.

R.M.MACKIE

Advanced Geriatric Medicine 6. Edited by F.I.CAIRD AND J.GRIMLEY EVANS. (1988) Bristol: Wright. Pp. 188. ISBN 0-7236-0651-X. £25.00.

I enjoyed reading this slim indexed hard-backed volume which is one of a series reviewing topics of relevance to the care of the elderly (i.e. over 65 age group). It has 19 contributors, most of whom work in Scotland. It is divided into four parts, namely, demography, dermatology, therapeutics and future developments. Maps, charts, graphs and statistics are plentiful in the non-dermatological sections and updated chapters on adverse drug reactions, hypothermia and choice of hypoglycaemic drug are always important.

The dermatology section occupies one-third of the book and contains the 17 monochrome illustrations. It is divided into five well-chosen chapters, namely, biology of ageing skin (Finlay), cutaneous infections (Cox and Roberts), blistering diseases (Harrison and Simpson), cutaneous malignancy (MacKie) and modern dressings for leg ulcers (Varghese and Carter). All are interesting and useful but a practical problem with modern dressings is that many are unavailable on NHS prescription. Both pemphigus foliaceus (p 101) and Dubreuilh (p 124) were wrongly spelt and the concentration of gamma benzene hexachloride (p 98) should surely be 1%.

The intended readership includes junior hospital doctors and recently appointed geriatricians. With this readership in mind the text suits admirably. It is a good book for the non-dermatologist, and the price is just about right.

J.VERBOV

The Vulva. Edited by C.M.RIDLEY. (1988) Edinburgh: Churchill Livingstone. Pp. 355. Price £60.

This superb book spans all that needs to be known about what can justly be called dermatology's last frontier. Because it is comprehensive, various chapters such as those on embryology and anatomy may sound uninteresting to the dermatological coterie. It would however be a mistake to ignore them since they provide a fascinating insight both into what comes in later chapters and also into those rather obscure problems of inter-sex syndromes which are normally so hard to understand.

The editor has distinguished herself with a concise overview of general dermatology as it relates to the vulva and then in a short chapter jointly written with a psychiatrist, she allows us a privileged insight into the female mind. Even more importantly she demolishes the notion (held by many) that pruritus vulvae is a common disease entity. Careful examination in good light will help differentiate many causes, for example, mild eczema, vulval microwarts and vestibulitis. Whilst vulvodynia and burning vulva syndrome, sympathetically defined and explained, account for virtually all the remainder.

The chapter on infective disease is perhaps too comprehensive but as a review of genito-urinary medicine it is to be highly rated; especially the section on wart virus infection. In this context it marries well with the last chapter on epithelial tumours of the vulva.

My major criticism is not of the book itself, but of the confusion surrounding the present classification of dysplastic vulval skin disease. The problem is historical in that dermatology's last frontier has been visited in earlier times by non-dermatological explorers. They introduced clinical and pathological 'place names' such as kraurosis and leukoplakia which even they found confusing. In an attempt to redefine the frontier everything was then renamed as a 'dystrophy'. This confused everyone so a group of classifiers has now come up with the concept of Vulval Intra-epithelial Neoplasia (VIN). The fact that this can be basaloid, Bowen's disease or Bowenoid and related to warts, makes it cumbersome, and impossible to include such entities as Paget's disease and malignant melanoma *in situ*. VIN I and VIN II are quoted as 'too poorly documented' to warrant clinical description and VIN III looks and sounds like Bowenoid papulosis. It is not good enough and time dermatology imposed a sense of order on this Tower of Babel.

That said, this book should be compulsory reading for all frontiersmen (and women) be they of the gynaecological or dermatological bent. Every department should buy a copy; mine is on loan to O & G!

F.A.IVZ

Books Received

TSTB. A New Diagnostic Method in Dermatological Practice. P.BERARDI. (1988) Fano: Editrice Fortuna Offset Stampa s.r.l.

Manual of Dermatologic Therapeutics—With Essentials of Diagnosis. K.A.ARNDT. (1988) 4th edition Boston: Little, Brown and Company. ISBN 0-316-051780. £9.95.

British Journal of Dermatology (1989) **120**, 722.

News and Notices

Finsen Medal

Professor Ian Magnus was awarded the Finsen Medal by the Association Internationale de Photobiologie at the 10th International Congress on Photobiology in Jerusalem. This award was made for outstanding work in Dermatological Photobiology by Professor Magnus.

International Symposium on Itch 18-19 May 1990 Stockholm

An international symposium 'Itch: basic and clinical aspects' will be held in Stockholm, Sweden, 18-19 May, 1990. The meeting is organized in collaboration with the Skin Pharmacology Society. Guest lectures will be presented by a faculty of leading neurophysiologists, neuropharmacologists and clinicians with a special interest in pain and itch. For further information, please contact: Dr Östen Hägermark, Department of Dermatology, Karolinska Sjukhuset, S-104 01 Stockholm, Sweden.

International Congress on Dermatology and Psychiatry, University of Leeds, England 9-11 July, 1989

The second International Symposium on Dermatology and Psychiatry will be held in the University of Leeds from 9 to 11 July, 1989. Topics include: Tactile hallucinations; Delusional parasitosis; Dysmorphophobia; Artefact Dermatitis; Psychiatric aspects of Tattoos and Psychoimmunology. Further details can be obtained from: Dr J.A. Cotterill, Department of Dermatology, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, Yorkshire, England.

British Journal of Dermatology (1989) **120**, 723.

Erratum

Sharquie KE, Al-Talib, Chu, AC. Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony. *British Journal of Dermatology* 1988; **119**, 54.

We regret that the numbers of patients treated in the summary and in the body of the text do not correlate in this paper. Therefore, starting at page 54, line 12, the text should read as follows: '30 lesions (20 dry, 10 wet) in patients receiving treatment were left as controls without any treatment. One hundred and thirty lesions (93 dry, 37 wet) were treated with Pentosam. These lesions were injected every 8 days and followed-up for 42 days after the last injection. The number of injections needed depended on the response of the particular lesion to treatment. One hundred and four lesions (81 dry, 23 wet) needed one injection and 20 lesions (10 dry, 10 wet) needed two injections. The remaining six lesions (two dry, four wet) required three injections.'

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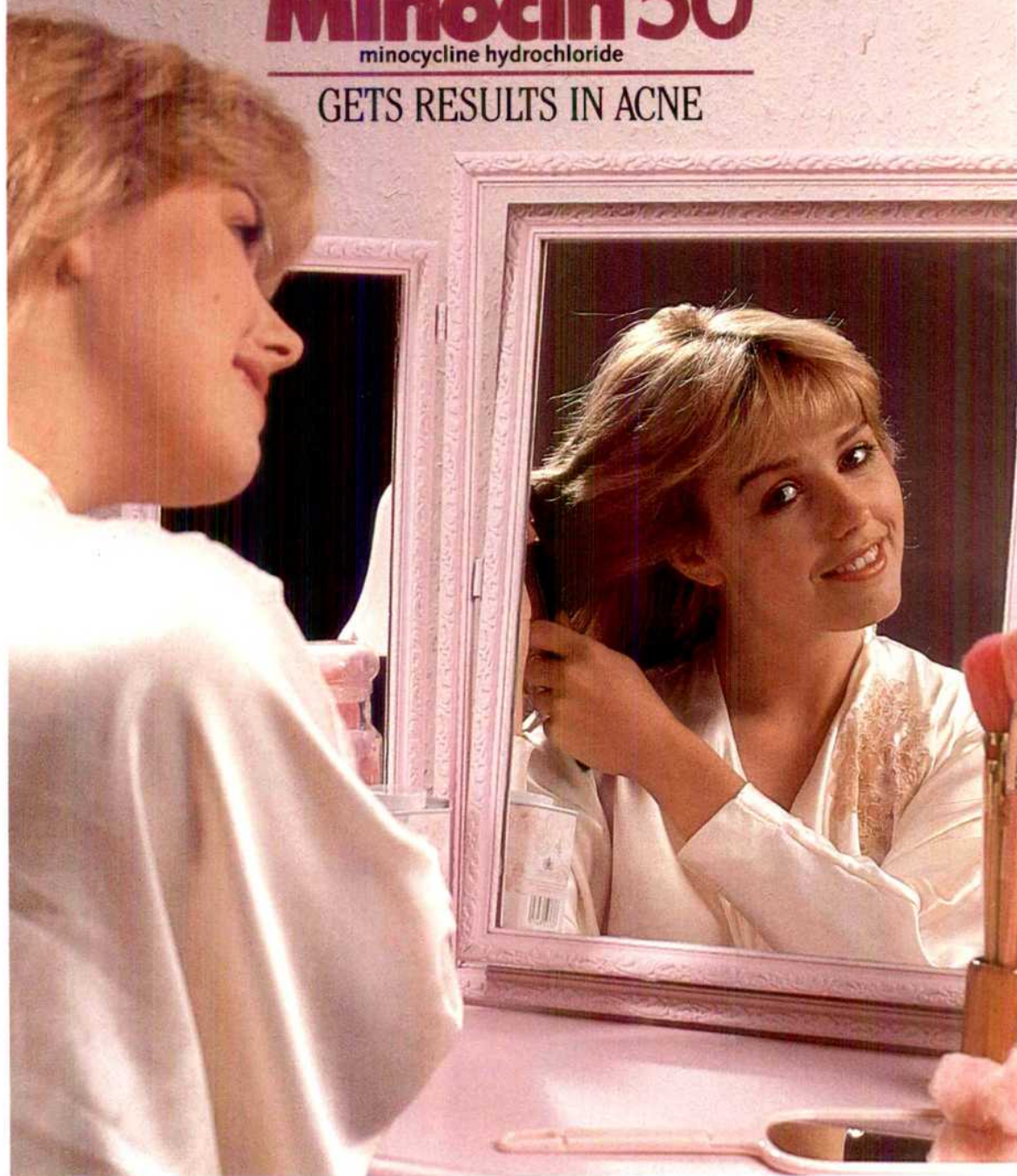
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microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometre(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	[¹³¹ I]
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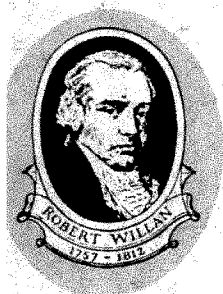
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CONTENTS	PAGE
Immediate decrease in antigen-presenting function and delayed enhancement of interleukin-1 production in human epidermal cells after <i>in vivo</i> UVB irradiation L.RÄSÄNEN, T.REUNALA, M.LEHTO, C.JANSÉN, I.RANTALA AND P.LEINIKKI	589
DNA analysis by flow cytometry in cutaneous T-cell lymphomas ELISABETH RALFKIAER, JØRGEN K.LARSEN, IB J.CHRISTENSEN, KRISTIAN THOMSEN AND GUNHILD LANGE WANTZIN	597
Peripheral blood mononuclear leukocyte cyclic adenosine monophosphate specific phosphodiesterase activity in childhood atopic dermatitis I.H.COULSON, S.N.DUNCAN AND C.A.HOLDEN	607
Keratinocyte expression of OKM5 antigen in inflammatory cutaneous disease J.N.W.N.BARKER, A.C.MARKEY, M.H.ALLEN AND D.M.MACDONALD	613
Calcitonin gene-related peptide immunoreactivity in prurigo nodularis: a comparative study with neurodermatitis circumscripta ANNIKKI VAALASTI, HANNA SUOMALAINEN AND LEENA RECHARDT	619
Human cutaneous mast cells—a study of fixative and staining reactions in normal skin A.C.MARKEY, LINDA J.CHURCHILL AND D.M.MACDONALD	625
Multiple infundibular tumours of the head and neck G.H.FINDLAY	633
Isotretinoin differs from other synthetic retinoids in its modulation of human cellular retinoic acid binding protein (CRABP) SUSANNE HIRSCHEL-SCHOLZ, G.SIEGENTHALER AND JEAN-HILAIRE SAURAT	639
An investigation into the incidence of auto-immune disorders in patients with localized morphoea CHRISTINE I.HARRINGTON AND I.R.DUNSMORE	645
Acne is not associated with abnormal plasma androgens M.J.LEVELL, M.L.CAWOOD, B.BURKE AND W.J.CUNLIFFE	649
Two novel techniques for the evaluation of barrier creams R.MARKS, P.J.DYKES AND I.HAMAMI	655
A double-blind study of topical 1 α , 25-dihydroxyvitamin D ₃ in psoriasis P.C.M.VAN DE KERKHOFF, M.VAN BOKHOVEN, M.ZULTAK AND B.M.CZARNETZKI	661
Combined treatment of psoriasis with acitretin and UVB phototherapy compared with acitretin alone and UVB alone J.IEST AND J.BOER	665
Familial keratoses of actinic distribution associated with internal malignancy and cellular hypersensitivity to UVA D.J.ATHERTON, P.K.BOTCHERBY, A.J.FRANCIS, N.P.SMITH, R.S.WELLS AND F.GIANNELLI	671
Cutaneous cryptococcosis: treatment with oral fluconazole D.SHUTTLEWORTH, C.M.PHILPOT AND A.G.KNIGHT	683
Non-symmetric subcutaneous lipomatosis associated with familial combined hyperlipidaemia A.RUBINSTEIN, Y.GOOR, E.GAZIT AND S.CABILI	689
Cutaneous chylous reflux L-C.CHERN, C-S.LIN AND C-K.WONG	695
Imported rickettsial African spotted fever in Japan N.ISHII, H.KOMATSU, H.NAKAJIMA, K.TANI AND T.SUTO	701
Prurigo pigmentosa successfully treated with minocycline M.ASO, T.MIYAMOTO, T.MORIMURA AND S.SHIMAO	705
Regression of oral Kaposi's sarcoma in a case of AIDS on Zidovudine (AZT) A.LANGFORD, B.RUF, R.KUNZE, H-D.POHLE AND P.REICHART	709
Correspondence	
Premenstrual exacerbation of atopic dermatitis D.KEMMETT	715
Purpura and sepsis—rarer causes? A.ZUMLA AND G.LIPSCOMB	715
Concomitant Becker's naevus and acanthosis nigricans R-F.H.J.HULSMANS AND F-J.H.HULSMANS	716
The use of water as a mounting medium for light microscopy to study the surface architecture of hair L.STANKLER	717
Book Reviews	719
News and Notices	722
Erratum	723



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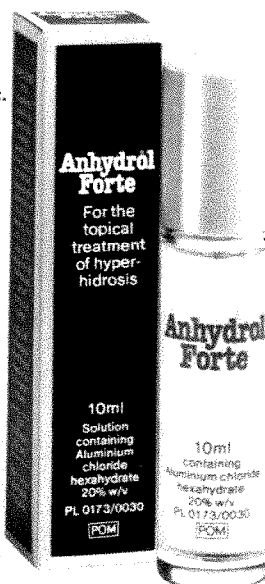
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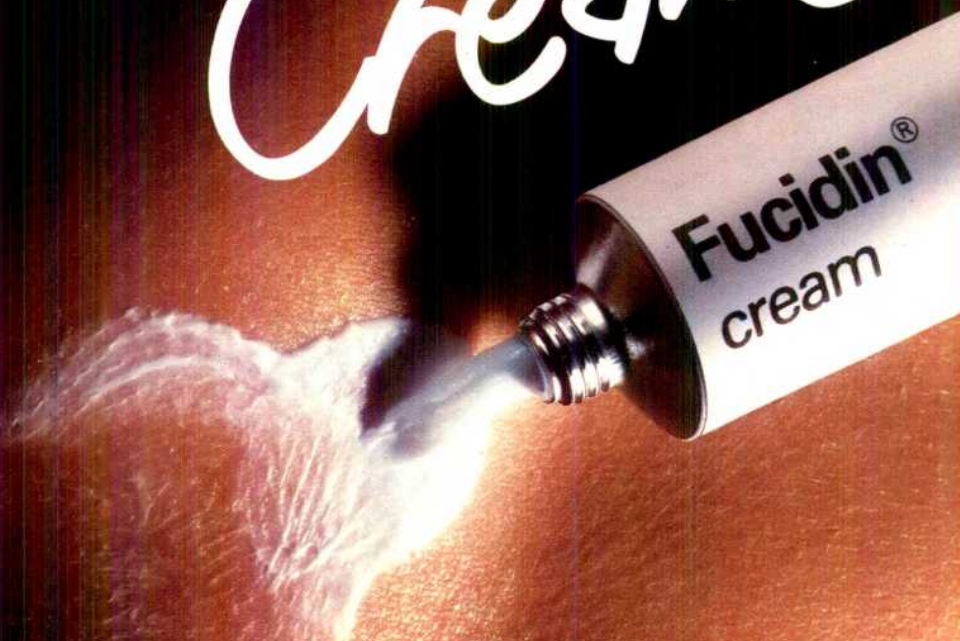
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- References**
1. Rapaport M., et al. *Cutis* **30**: 122-135 (1982)
 2. Bernstein MD., et al. *J. Am. Acad. Dermatol.* **4**: 318-321 (1980)
 3. Eady EA., et al. *J. Am. Acad. Dermatol.* **4**: 55-56 (1981)
 4. Rivkin I., Rapaport M. *Cutis* **25**: 552-55 (1980)

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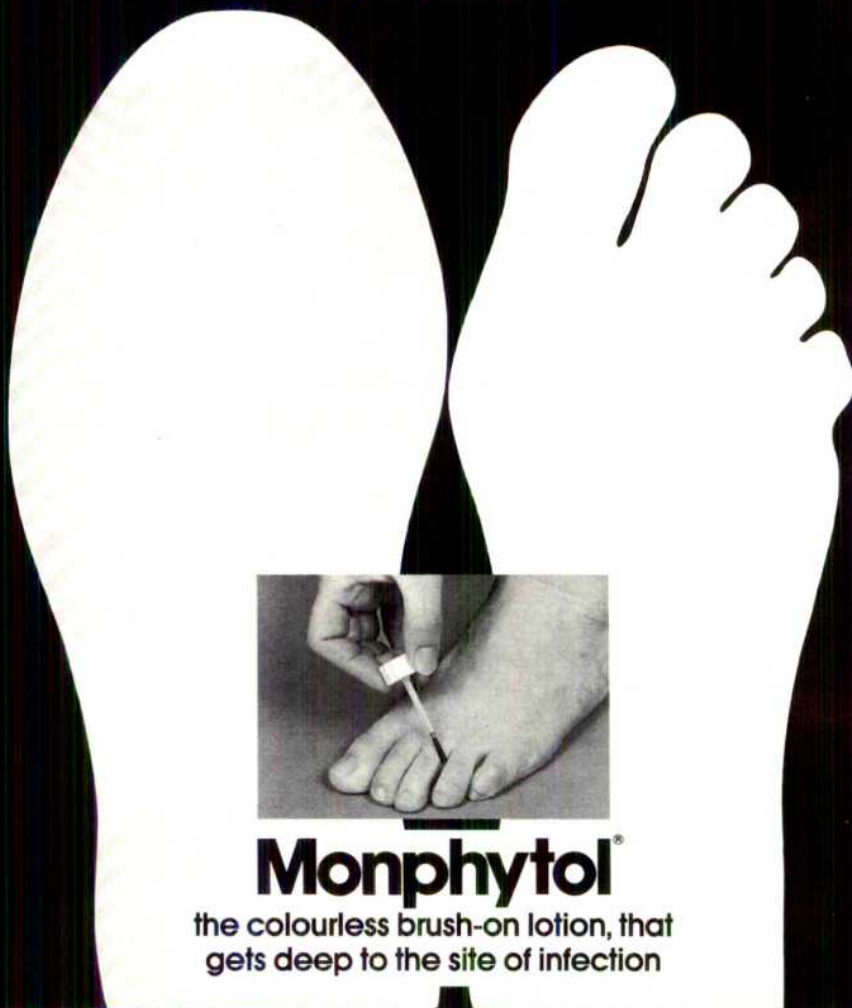


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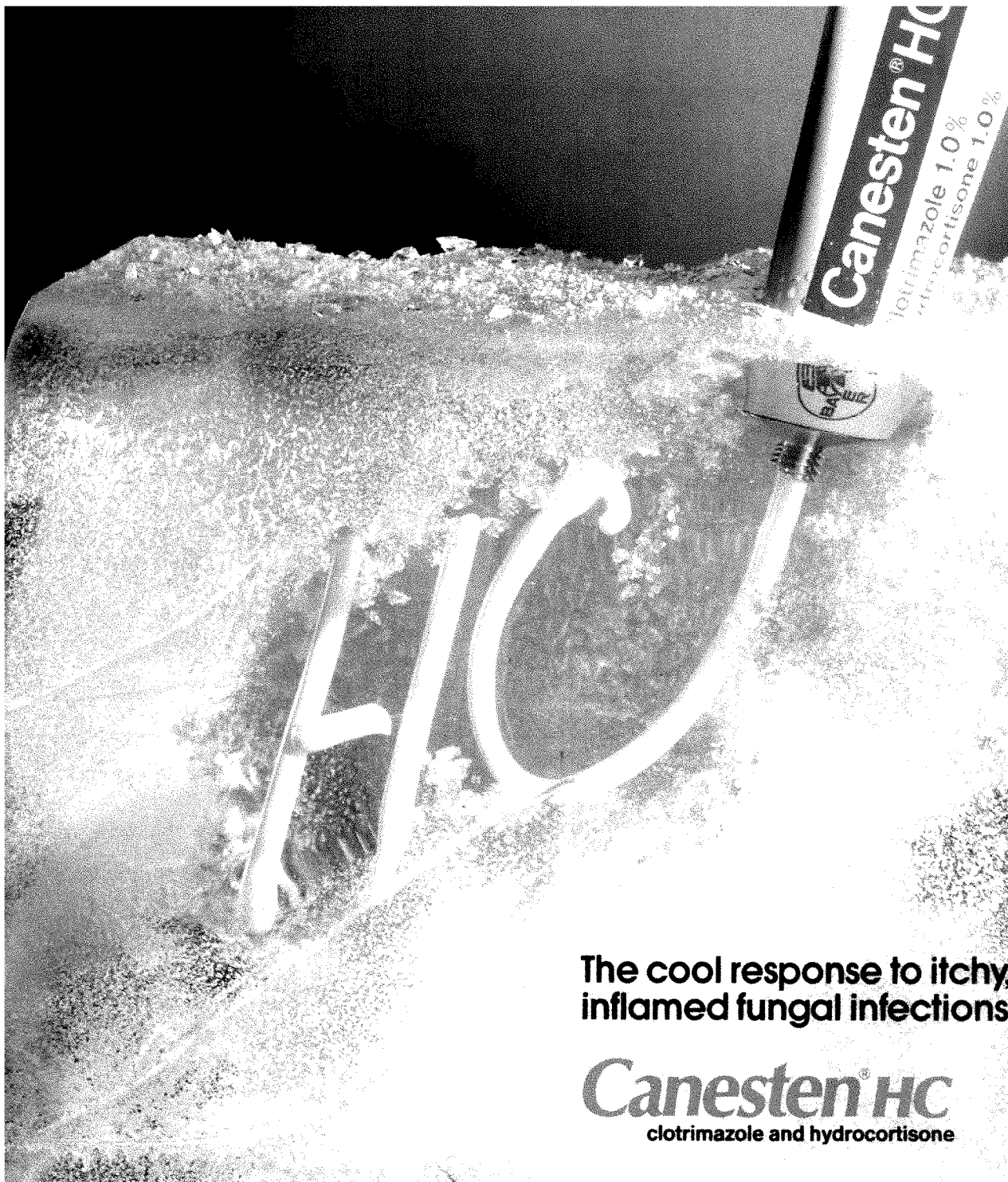
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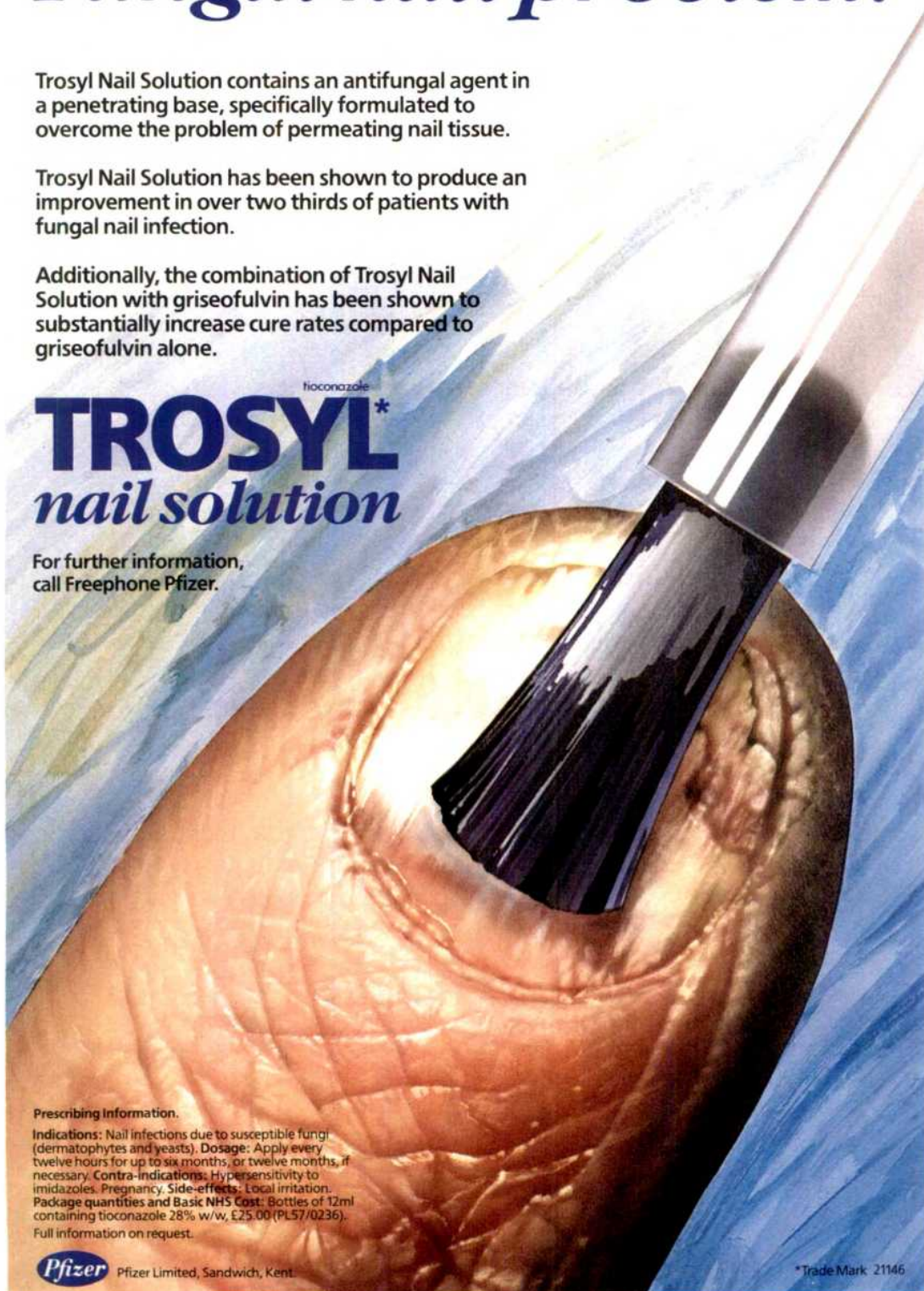
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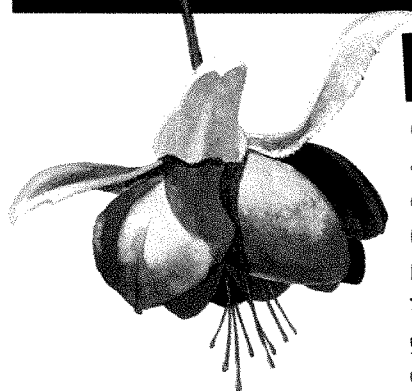
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1. Bunney, M.H. et al (1976) - Br. J. Derm. **94** 667-697

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Vitronectin colocalizes with Ig deposits and C9 neoantigen in discoid lupus erythematosus and dermatitis herpetiformis, but not in bullous pemphigoid

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Accepted for publication 19 December 1988

SUMMARY

C9 neoantigen immunoreactivity has been found to colocalize with C3 immunoreactivity at the dermal-epidermal junction zone (DEZ) in skin specimens from patients with bullous pemphigoid, lupus erythematosus and dermatitis herpetiformis. The present study was designed to elucidate whether the C9 neoantigen immunoreactivity represents deposition of membrane attack complexes or non-lytic SC5b-9 complexes. Skin specimens from 11 patients with pemphigoid, five patients with discoid lupus erythematosus and from nine patients with dermatitis herpetiformis were studied with immunofluorescence using both monoclonal and polyclonal antibodies against C9 neoantigen and against vitronectin (S-protein), an inhibitor to the membrane attack complex of complement. Specimens from the pemphigoid patients demonstrated C9 neoantigen reactivity along the DEZ without detectable colocalized vitronectin. This suggests deposition of membrane attack complexes in the pemphigoid lesions. Immunoreactivity of both C9 neoantigen and vitronectin was detected in the DEZ in specimens of discoid lupus erythematosus and in the tips of dermal papillae in specimens of dermatitis herpetiformis. The combined presence of C9 neoantigen- and vitronectin immunoreactivity may indicate deposition of C9 as part of the non-lytic SC5b-9 complex. The finding reported here of differential deposition of vitronectin and C9 in different diseases indicates that the presence of C9 neoantigen immunoreactivity in tissue *per se* does not represent the deposition of membrane attack complexes, but that it may also be C9 deposited as part of the nonlytic SC5b-9 complex.

The characteristic gamma globulin- and/or C3-immunofluorescence patterns, found in skin specimens from patients with bullous pemphigoid, dermatitis herpetiformis or discoid lupus erythematosus, are widely used in diagnosis.¹

Correspondence: Dr Karin Dahlbäck, Department of Dermatology, University of Lund, University Hospital, S-22185 Lund, Sweden.

In bullous pemphigoid a linear deposition of IgG and C3 is found at the DEZ and activation of complement is believed to be of importance in the pathogenesis of this disease.^{2,3} The patients' sera frequently contain autoantibodies directed against the bullous pemphigoid antigen, a glycoprotein found to be associated with hemidesmosomes within basal keratinocytes and in the lamina lucida space outside the cells. Results from several studies suggest that such autoantibodies may activate the complement system, primarily via the classical pathway, leading to release of fragments with anaphylatoxic and chemotactic activities. Proteolytic enzymes liberated from attracted leucocytes are thought to be involved in blister formation. A direct cytolytic effect of the membrane attack complex (MAC) of complement, leading to lysis of basal keratinocytes has also been proposed.⁴

In dermatitis herpetiformis, a granular or fibrillar pattern of IgA- and C3-immunoreactivity in the tips of the dermal papillae is characteristic, both in normal and lesional skin. In the lesional skin of discoid lupus erythematosus, a broad band (referred to as the 'lupus-band') of IgG- and/or IgM- and C3-immunoreactivity, is usually found along the DEZ. In spite of the clinical usefulness of the detection of C3 and gamma globulins in the skin of patients with these disorders, the role of complement in the pathogenesis of skin lesions in dermatitis herpetiformis and discoid lupus erythematosus is unclear.^{1,5}

Activation of the complement system on a cell surface leads to the assembly of the complement proteins, C5-C9, in the formation of the membrane attack complex (MAC) which causes cell lysis by C9 polymerization.⁶ In fluid phase, vitronectin binds to nascent C5b-7 complexes, thereby inhibiting the C9 polymerization and the membrane attack.^{7,8} Thus, complement activation in the absence of a phospholipid membrane leads to the formation of a non-lytic complex composed of vitronectin (S-protein) and C5b-9, the so-called SC5b-9 complex. Both during the assembly of MAC and of SC5b-9, a major molecular rearrangement occurs in C9 leading to the exposure of neoantigens.^{9,10}

Vitronectin exists not only in plasma but also in tissue.^{11,12} In skin we have demonstrated vitronectin immunoreactivity in association with elastic fibres in adults.^{12,13} At present it is unclear whether the tissue form of vitronectin, like its fluid phase counterpart, has an inhibitory effect on the terminal complement pathway.

Antibodies against C9 neoantigens have been used to demonstrate C9 immunoreactivity in specimens from patients with bullous pemphigoid, dermatitis herpetiformis and discoid lupus erythematosus.^{4,14,15} In bullous pemphigoid, C9 neoantigen was found in a linear pattern at the DEZ in both involved and uninvolved skin, the amount being greater in involved skin as judged by intensity of immunofluorescence.⁴ In dermatitis herpetiformis, C9 neoantigen was present in the tips of dermal papillae in uninvolved skin (involved skin was not studied).¹⁴ Both in discoid and in systemic lupus erythematosus, C9 neoantigen immunoreactivity was found as a lupus band along the DEZ in involved skin only.¹⁵

Antibodies specific for the C9 neoantigens do not react with native C9, nor do they distinguish between C9 in MAC and C9 in the SC5b-9 complex. Thus, it may be incorrect to interpret the presence of C9 neoantigen immunoreactivity in the tissue as an indication of deposited MAC. Instead it may be due to deposition of SC5b-9 complexes. To elucidate this, we have now correlated the C9 neoantigen immunoreactivity with that of vitronectin (S-protein) in skin biopsies from patients with bullous pemphigoid, dermatitis herpetiformis and discoid lupus erythematosus. The results suggest deposition of membrane attack complexes in lesions of pemphigoid, whereas in discoid lupus erythematosus and in dermatitis herpetiformis, the combined presence of vitronectin and C9 may suggest deposition of nonlytic SC5b-9 complexes.

METHODS

Skin specimens were studied from patients with clinical and immunohistochemical signs of bullous pemphigoid (11 cases), dermatitis herpetiformis (nine cases) and discoid lupus erythematosus (five cases). The specimens were surplus material from biopsies referred to the laboratory for diagnostic immunofluorescence examination from 10 different hospitals in Sweden. The specimens from patients with pemphigoid were from perilesional skin. The specimens from patients with dermatitis herpetiformis were from perilesional skin (three specimens) and from normal skin (six specimens). The specimens from patients with discoid lupus erythematosus were from skin lesions. The relevant clinical data were obtained and only cases with typical clinical features were included in the study. Diagnostic immunofluorescence demonstrated a linear band of C3 and IgG along the DEZ in the specimens from patients with bullous pemphigoid. The patients with dermatitis herpetiformis had typical granular IgA- and C3-reactivity in the tips of dermal papillae. The cases with discoid lupus erythematosus showed C3 and IgM and/or IgG in a pattern of a lupus band at the dermal-epidermal junction.

Fixation procedure

The specimens were immersed in a transport medium (550 g ammonium sulphate added to 1 litre 25 mM potassium citrate, 5 mM *N*-ethylmaleimide, 5 mM magnesium sulphate), washed within 48 h in transport medium lacking ammonium sulphate, and then frozen in chlordifluoromethane R22 at the temperature of liquid nitrogen. The specimens were stored at -70°C until processed. Cryostat sections were between 4 and 10 μm thick. They were air-dried for 30 min before being incubated with the primary antibodies.

Proteins and primary antisera

A specific rabbit polyclonal anti-vitronectin antiserum was used (previously characterized in detail).¹² A monoclonal anti-vitronectin antibody was the kind gift of Dr John Tamerius at Cytotech, San Diego, CA, U.S.A. Both in this and earlier studies, the results obtained with the polyclonal vitronectin antiserum demonstrated an excellent correlation with those obtained with monoclonal anti-vitronectin.^{13,16,17}

A polyclonal antiserum specific for C9 neoantigens was used (previously characterized in detail).¹³ Monoclonal antibody against C9 neoantigen was the kind gift of Dr T.E.Mollnes, Department of Immunology, Rikshospitalet, Oslo, Norway.¹⁸ The results obtained with the polyclonal C9 neoantigen antiserum, both in this and an earlier study, demonstrated an excellent correlation with those obtained with the monoclonal anti-C9-neoantigen.¹³ The working dilutions used in the immunofluorescence experiments were 1:3000 (monoclonal anti-C9 neoantigen), 1:100 (polyclonal anti-C9 neoantigen), 1:500 (monoclonal anti-vitronectin) and 1:4000 (polyclonal anti-vitronectin). In the avidin-biotin peroxidase complex technique the working dilution of anti-C9 neoantigen was 1:30000.

Fluorescein-conjugated (FITC) rabbit anti-human C3c, anti-human IgG, anti-human IgA, anti-human IgM, FITC swine anti-rabbit IgG and FITC rabbit anti-mouse IgG were from Dakopatts a/s Copenhagen, Denmark. Biotinylated horse anti-mouse Ig were from Vector Laboratories, Burlingame, CA, U.S.A.

Immunohistochemical techniques

Cryostat sections were incubated with their respective primary antisera for 30 min. When a

double layered technique was used, the sections were subsequently washed and then incubated with FITC swine anti-rabbit IgG or FITC rabbit anti-mouse IgG for 30 min before examination in the fluorescence microscope.

To demonstrate colocalization of IgA with C9 in the granules in dermal papillae, a sequential staining procedure was used on some specimens from patients with dermatitis herpetiformis as follows: in the first reaction, FITC rabbit anti-human IgA was used in the immunofluorescence technique. After photography, the specimens were then processed with monoclonal anti-C9 neoantigen in the avidin-biotin peroxidase complex technique,¹⁹ using biotinylated horse anti-mouse Ig as the secondary antiserum.

In a similar sequential immunostaining procedure, colocalization of C9 and vitronectin in the granules in dermal papillae of dermatitis herpetiformis patients was demonstrated as follows: in the first reaction a double layered immunofluorescence technique was used with polyclonal anti-vitronectin as the primary antibody and FITC swine anti-rabbit Ig as the secondary antibody. After photography, the specimens were processed with monoclonal anti-C9 neoantigen in the avidin-biotin peroxidase complex technique, using biotinylated horse anti-mouse Ig as secondary antiserum.

As negative controls, buffer or unrelated antibodies (polyclonal anti-amyloid A antibodies) were used.

RESULTS

In the specimens from the patients with bullous pemphigoid both C3- and IgG-immunoreactivities were found as bright, thin, linear bands along the DEZ. Immunoreactivity of C9 neoantigen was also detected in these specimens, but the pattern differed from that of C3 immunoreactivity in 10 of the 11 specimens. The C9 neoantigen immunoreactivity either presented as a continuous or as an intermittent granular band at the DEZ (Fig. 1a) and only one specimen showed a linear band of C9 neoantigen immunoreactivity, similar to that of C3. The results obtained with polyclonal and monoclonal C9 neoantigen antibodies were identical. No vitronectin immunoreactivity was detected at the DEZ in 10 of the 11 specimens (Fig. 1b). The one specimen which had linear C9 neoantigen immunoreactivity also had a faint linear band of vitronectin immunoreactivity in the same zone.

In dermatitis herpetiformis, granules in the tips of the dermal papillae stained with anti-IgA and anti-C3. In all specimens a positive granular immunoreactivity of C9 neoantigen and vitronectin with a similar distribution was observed (Fig. 2). Using sequential staining procedures, it was demonstrated that the granular papillary structures that stained with the anti-IgA antiserum also stained with the anti-C9 neoantigen antibody (Fig. 3) and that the staining patterns obtained with anti-C9 neoantigen and anti-vitronectin were essentially identical (Fig. 4), suggesting co-localization of the three proteins.

In all specimens from patients with discoid lupus erythematosus, C3- and IgG- and/or IgM-immunoreactivity could be demonstrated in the pattern of a lupus band at the DEZ. Essentially identical patterns were obtained both with anti-C9 neoantigen and with anti-vitronectin (Fig. 5).

In normal adult skin, vitronectin is associated with the elastin fibres,¹² and this was also found in all specimens studied in this investigation. Faint C9 immunoreactivity associated with the elastic fibres was observed in some specimens in this investigation as had been found in other studies.¹³

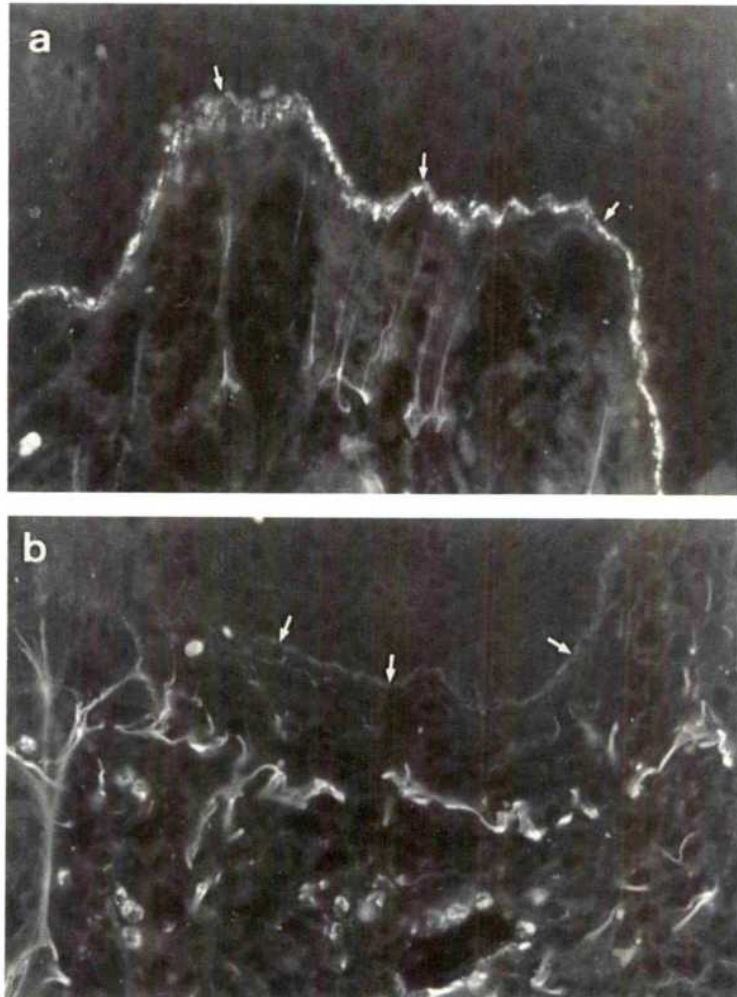


FIGURE 1. Immunostaining of skin lesion from a patient with bullous pemphigoid with anti-C9 neoantigen and anti-vitronectin. Sections of a skin specimen from a patient with bullous pemphigoid examined with immunofluorescence using (a) polyclonal anti-C9 antiserum and (b) polyclonal anti-vitronectin antiserum. Note the granular C9 immunoreactivity in the DEZ. Using anti-vitronectin, the area of the DEZ was unstained, but the dermal elastic fibres showed distinct immunofluorescence. ($\times 335$).

DISCUSSION

The presence of C9 neoantigen immunoreactivity in lesions of bullous pemphigoid, dermatitis herpetiformis, and discoid lupus erythematosus has been considered to indicate deposition of membrane attack complex.^{4,14,15} However, since the presence of vitronectin (S-protein) immunoreactivity was not examined, it was uncertain whether the C9 neoantigen immunoreactivity represented deposition of membrane attack complexes or non-lytic SC5b-9 complexes. To study potential deposition of terminal complement complexes in tissue, C9 neoantigen and

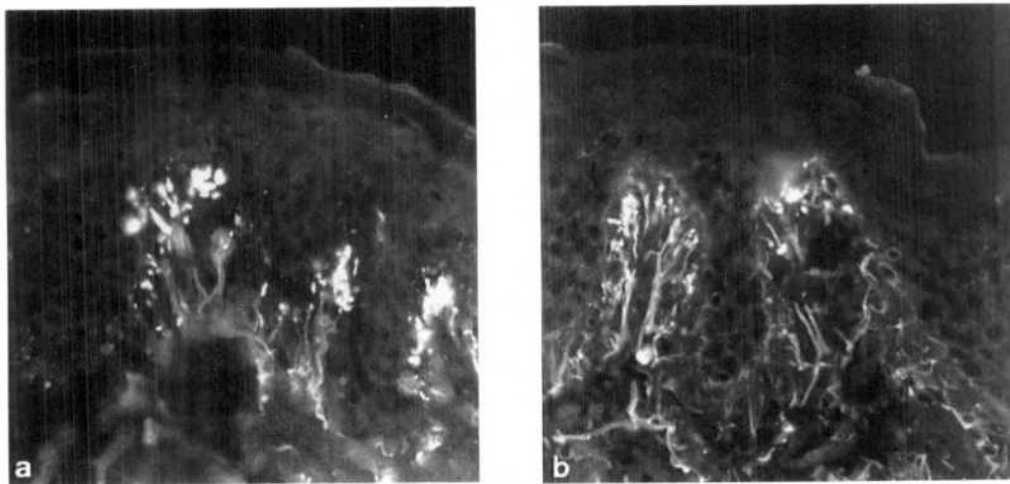


FIGURE 2. Immunostaining of skin lesion from a patient with dermatitis herpetiformis with anti-C9 neoantigen and anti-vitronectin. Sections of a skin specimen from a patient with dermatitis herpetiformis, examined with immunofluorescence using (a) polyclonal anti-C9 antiserum and (b) polyclonal anti-vitronectin antiserum. Note the granules showing distinct immunofluorescence. ($\times 340$).

vitronectin immunoreactivity should preferably be examined in parallel. There are only a few other reports where this approach has been adopted.^{18,20-22}

In bullous pemphigoid we have now found deposition of C9 neoantigen, but no vitronectin along the DEZ in 10 out of 11 specimens. This suggests the presence of MAC rather than of SC5b-9 complexes, and supports the hypothesis of a direct lytic action of MAC on basal keratinocytes,⁴ possibly contributing to the formation of bullae in bullous pemphigoid. Our finding of a granular rather than linear pattern of C9 neoantigen immunoreactivity along the

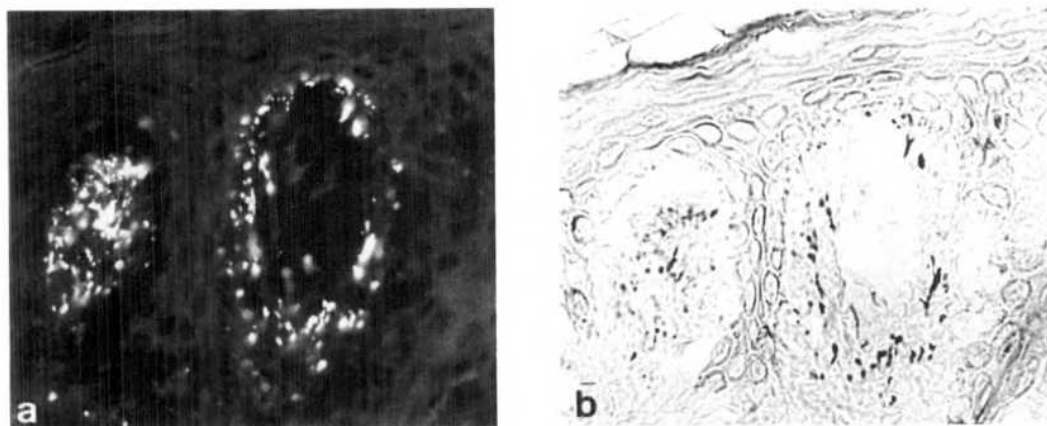


FIGURE 3. Sequential immunostaining of skin lesion from a patient with dermatitis herpetiformis demonstrating colocalization of immunoreactivity of IgA and C9 neoantigen. A section of a skin specimen was immunostained (a) with FITC rabbit anti-human IgA in an immunofluorescence technique, and (b) with monoclonal anti-C9 neoantigen in the avidin biotin peroxidase complex technique. ($\times 450$).

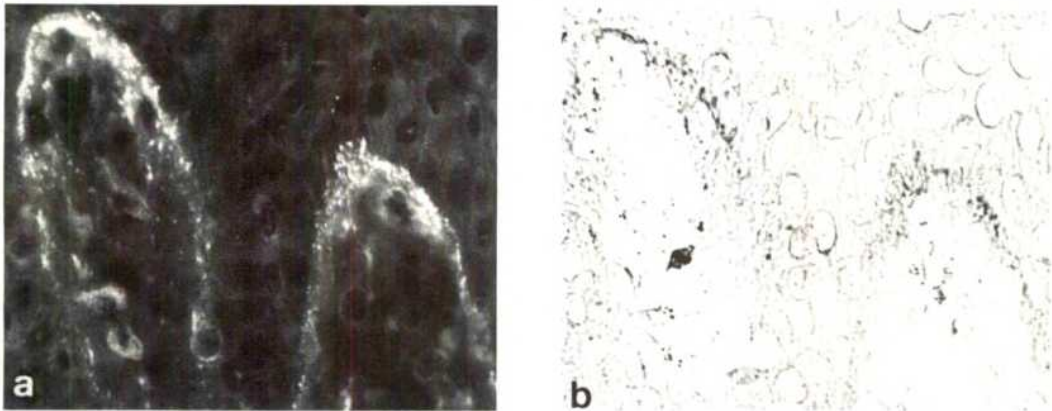


FIGURE 4. Sequential immunostaining of skin lesion from a patient with dermatitis herpetiformis demonstrating colocalization of immunoreactivity of vitronectin and C9 neoantigen. A section of a skin specimen from a patient with dermatitis herpetiformis was immunostained (a) with polyclonal anti-vitronectin in an immunofluorescence technique and (b) with monoclonal anti-C9 neoantigen in the avidin-biotin peroxidase complex technique. ($\times 450$).

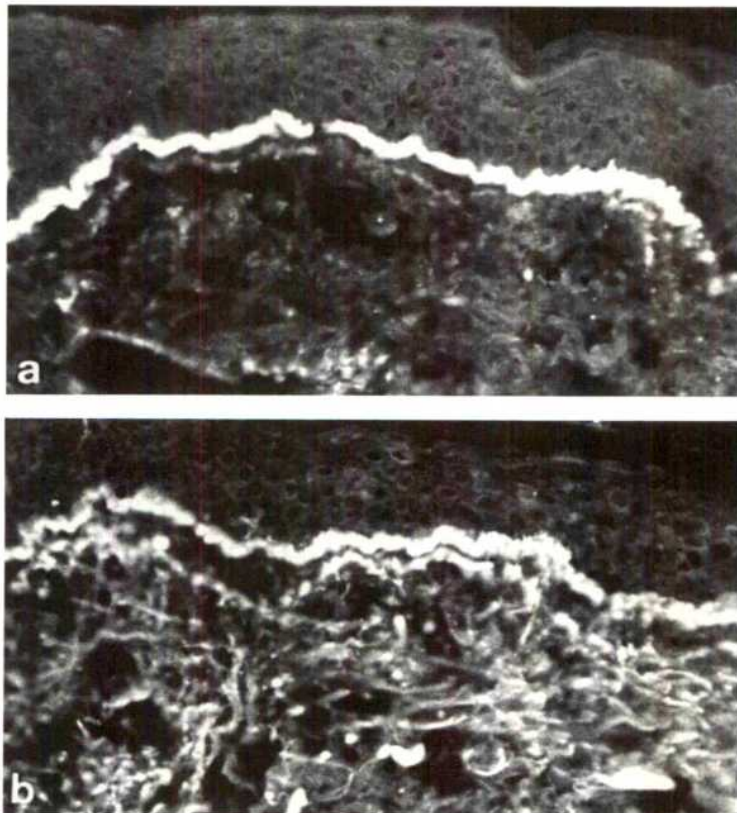


FIGURE 5. Immunostaining of skin lesions from a patient with discoid lupus erythematosus with anti-C9 neoantigen and anti-vitronectin. Sections of a skin specimen from a patient with discoid lupus erythematosus examined with immunofluorescence using (a) polyclonal anti-C9 antiserum and (b) polyclonal anti-vitronectin antiserum. Note the thick lupus band seen with both antisera. ($\times 265$).

DEZ in all specimens except one is at variance with an earlier study, where the pattern of C9 neoantigen reactivity was seen as a linear band, indistinguishable from the immunostaining patterns of anti-IgG and anti-C3.⁴

Very few reports are available where selective C9 neoantigen deposition has been demonstrated without colocalized vitronectin in injured tissue. It has been found in vessel walls of inflamed urinary bladder from a patient with 'SLE-like autoimmune disease' and in infarcted areas of the myocardium.^{18,22} The findings in bullous pemphigoid presented here prompted our further investigation of C9 neoantigen and vitronectin in dermatitis herpetiformis and discoid lupus erythematosus.

In contrast to our findings in bullous pemphigoid, colocalization of C9 neoantigen and vitronectin immunoreactivity was seen in dermatitis herpetiformis and in discoid lupus erythematosus. This suggests deposition of non-lytic SC5b-9 complex rather than of MAC in these locations. Similar findings of colocalized C9 neoantigen and vitronectin have been reported in cases of several different types of nephritis, including lupus nephritis and IgA nephritis, and in vessel walls in the inflamed colon of patients with ulcerative colitis.^{20,21}

The occurrence of C9 neoantigens together with vitronectin in tissue may reflect *in situ* formation of SC5b-9 complexes or deposition of fluid phase SC5b-9 complexes. The vitronectin immunoreactivity found here at the DEZ in patients with dermatitis herpetiformis or discoid lupus erythematosus is not found in skin from healthy subjects. In normal adults vitronectin immunoreactivity is found only on elastic fibres.¹² This suggests that the vitronectin immunoreactivity now found in granules in tips of dermal papillae of patients with dermatitis herpetiformis and in the 'lupus band' in the skin of patients with discoid lupus erythematosus does not represent normal tissue vitronectin, but rather may reflect deposition of fluid-phase SC5b-9 complexes.

The role of IgA in dermatitis herpetiformis is not understood. The granular deposits of IgA immunoreactivity in the dermal papillae have been demonstrated ultrastructurally as amorphous substances in conjunction with elastin-associated and other microfibrils of 10 nm diameter.^{5,23} Interestingly, in normal adult skin, vitronectin immunoreactivity has been localized ultrastructurally to the periphery of elastic fibres, in close association with such microfibrils.¹³ The similar findings of immunoreactivity of C9 neoantigen and vitronectin both in normal and lesional skin implies that the deposition of these antigens is not involved in the pathogenesis of the lesions in dermatitis herpetiformis.

In lupus erythematosus, the role of complement and the pathogenetic significance of the lupus band is unclear. Biezecker *et al.*¹⁵ found C9 neoantigen immunoreactivity in lesional skin only, and not in normal skin in patients with lupus erythematosus, whereas C3 was present both in normal and lesional skin, suggesting a pathogenetic involvement of the terminal complement complex in the formation of lesions in lupus erythematosus. Our results indicate that the C9 neoantigen immunoreactivity reflects presence of SC5b-9 complexes associated with immune complexes along the DEZ.

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Immunopathology of the placenta in pemphigoid gestationis and linear IgA disease

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SUMMARY

We have investigated the immunopathology of the placenta in bullous diseases by studying the deposition of immune complexes and expression of MHC class II subregion products by immunohistological methods. Placentae from seven patients with pemphigoid gestationis (PG) and two patients with linear IgA disease were studied. In PG immune complexes containing IgG1 and C₃ were identified in six cases. In linear IgA disease IgA1 containing immune complexes were found in both cases. Placentae from patients with PG showed aberrant expression of MHC Class II products. This was not seen in the placentae from patients with linear IgA disease. In PG there was incoordinate expression of the subregion products, DP and DR being more extensively and consistently expressed than DQ. These results and previous immunogenetic studies suggest that PG may be unique among organ specific autoimmune disease, the autoantibodies forming during an allogeneic response rather than target cells behaving as antigen presenting cells.

Pemphigoid gestationis (PG) is an acquired subepidermal blistering disorder most frequently associated with pregnancy.¹ Kelly *et al.*² have shown that in the serum of patients with PG there is an autoantibody which reacts with a basement membrane zone (BMZ) antigen in both skin and placenta. The antigen is first expressed in the placenta during the second trimester coinciding with the peak time of onset of the disease. The major antigen in PG has recently been shown to be a 180 kDa glycoprotein found in the epidermal BMZ.³

The association with pregnancy and the identification of cross reactivity between placenta and skin in PG suggests that the autoimmune response may be initiated by the placenta.² Initiation of immune responses in several autoimmune diseases has been shown to be associated with aberrant expression of MHC Class II molecules in the target tissues.⁴ The expression of MHC Class II molecules in such tissues has been studied using monoclonal antibodies and there are now available monoclonal antibodies specific for the products of the different subregions of the MHC.⁵

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Using direct immunofluorescence methods we have studied the deposition of immune complexes in the placentae of seven patients with PG and two patients with linear IgA dermatosis (LAD) who became pregnant during the course of their disease. We have also investigated the expression of MHC Class II molecules in the placentae of five PG patients and two patients with LAD using monoclonal antibodies and an immunoperoxidase method.

METHODS

Patients

Seven patients were diagnosed as having PG and two as having LAD by clinical, histopathological and immunohistopathological criteria.⁶

Tissues

Placentae from the patients and from five normal patients with no evidence of skin disease were obtained at delivery. Placentae from five therapeutic terminations at 12–16 weeks gestation and from five spontaneous abortions at 22–26 weeks gestation were also obtained. Small tissue blocks from all placentae (1 cm diameter) were snap frozen in liquid nitrogen and stored at -70°C until use. Sections 6 μm thick were cut and stained with haematoxylin and eosin. Multiple adjacent sections were cut and stained by immunoperoxidase or immunofluorescence methods.

Immunofluorescence

Polyclonal FITC labelled rabbit anti-human IgG, anti-IgA and anti-C₃ were obtained from Dako (High Wycombe, Bucks, U.K.)

Murine monoclonal antibodies specific for each of the IgG and IgA subclasses were obtained commercially. The specificity details of these have been described previously.⁶ Cryostat sections of placenta were stained using these reagents in a standard direct immunofluorescence protocol⁷ modified for use with the monoclonal antibodies.⁶ Sections were mounted in buffered glycerol and examined by UV microscopy.

TABLE 1. Monoclonal antibodies against MHC class II products and immunoglobulin subclasses

Monoclonal antibody	Specificity	Reference
DA 6-231	DP + DQ + DR	22
DA 6-147	DR + DQ	22
B7/21	DP	23
DA 6-164	DR	24
Leu 10	DQ	25
Tu 22	DQ	26
NL 16	IgG1	6
GOM 2	IgG2	6
ZG 4	IgG3	6
RJ 4	IgG4	6

Control sections were incubated with normal rabbit serum, normal mouse serum or an irrelevant monoclonal antibody preparation replacing the test serum in the immunofluorescence procedure.

Immunoperoxidase

Murine monoclonal antibodies to different products of the human MHC Class II subregions were used. The antibody names and specificity details are summarized in Table 1.

Cryostat sections of the placentae were cut, fixed in acetone for 10 min, air-dried and stained by a standard indirect immunoperoxidase method using peroxidase labelled anti-mouse IgG (Dako) as the second layer. Antibody binding was visualized by the peroxidase controlled precipitation of the diamino benzidine reaction product.

Control sections were incubated with normal mouse serum replacing the monoclonal antibodies. A cryostat section of tonsil provided a positive control for each experiment.

RESULTS

Morphology

Examination of the haematoxylin and eosin stained sections showed a focal mild increase in the cellularity of the amniochorionic stroma in 5/7 of the placentae from PG patients. There was no evidence of lesional separation of the amnion or chorionic epithelia. All the remaining placentae were morphologically normal.

Demonstration of immune deposits in the placentae

Pemphigoid gestationis. In three placentae from patients with PG we detected IgG using the polyclonal antiserum. When the IgG subclass specific monoclonal antibodies were used, in six cases IgG1 was detected (Fig. 1). One case also showed deposition of IgG2 and IgG3. In all

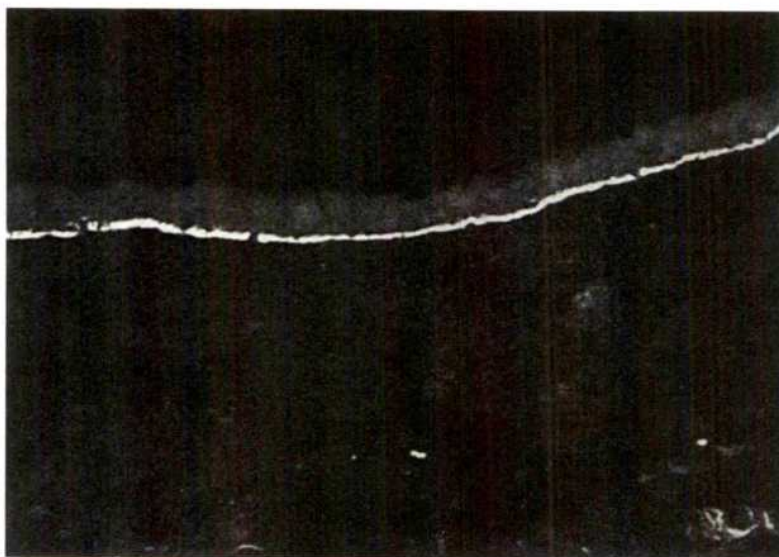


FIGURE 1. A linear band of fluorescence in the placental BMZ in a case of PG stained with anti IgG1. Placenta, pemphigoid gestationis. Immunofluorescence.

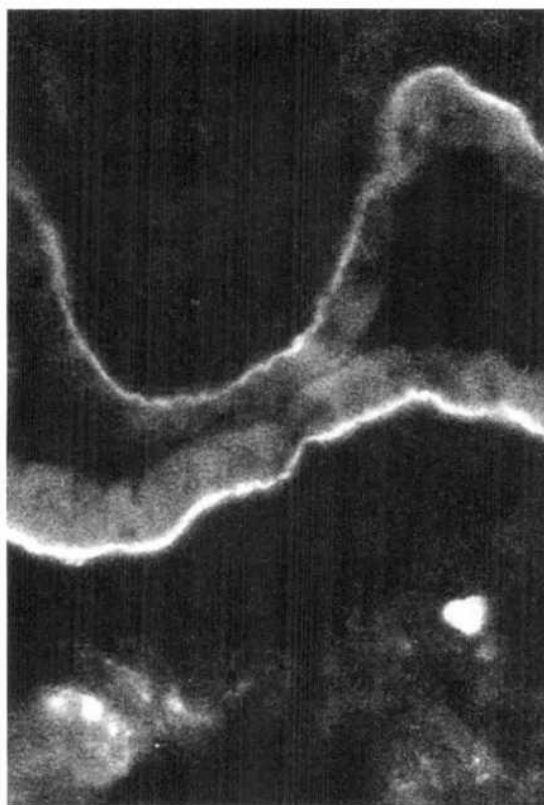


FIGURE 2. IgA1 staining of the placental BMZ in a case of LAD. Placenta, linear IgA dermatosis. Immunofluorescence.

examples the immunoreagents were visualized as a bright linear fluorescence at the BMZ of the amnion or chorionic epithelium. C_3 was also detected at this position in six cases. No reactivity for IgA or either of the IgA subclass epitopes were found.

Linear IgA diseases. In both cases the polyclonal anti IgA antiserum showed a bright fluorescent band at the BMZ of the amnion and chorionic epithelium. Subclass analysis showed that in both cases only IgA1 (Fig. 2) was detected. No reactivity for IgG or IgG subclasses was seen in these cases and no complement fixation was identified.

These results are summarized in Table 2.

Distribution of MHC II subregion products in normal placentae

In the 12–16 week placentae sparsely distributed spindle shaped stromal cells showed expression of MHC Class II molecules. There was staining with DA 6·231 and B7/21 (Fig. 3). There was no staining with the other antibodies.

At 22–26 weeks gestation, cells of a similar morphology stained with DA 6·231, DA 6·164, DA 6·147 and B7/21. Again these cells were infrequently present. No staining with Leu 10 or Tu 22 was observed.

TABLE 2. Antibody deposition in placentae

	IgG	IgG1	IgG2	IgG3	IgG4	IgA	IgA1	IgA2
Normal	—	—	—	—	—	—	—	—
Pemphigoid gestationis (n=7)	3	6	1	1	—	—	—	—
Linear IgA disease (n=2)	—	—	—	—	—	2	2	—

In full term placentae these occasional stromal cells stained with all the monoclonal antibodies against MHC Class II subregion products (Fig. 3).

Pemphigoid gestationis. Five placentae were studied for MHC Class II subregion product expression. The sparse population of spindle shaped cells in the amniochorionic stroma stained with all the anti MHC II antibodies. In addition, large numbers of polygonal cells in the stroma, predominantly in the chorionic villi but also underlying the amnion, showed MHC class II expression. These cells showed strong staining with DA 6·231, DA 6·164, DA 6·167 and B7/21 (Fig. 4). Focal staining with DA 6·231, DA 6·164 and DA 6·167 was seen in the trophoblast in four cases. No staining was observed with Leu 10 and only an occasional cell of this population stained with Tu 22.

Linear IgA disease. Both placentae showed MHC Class II subregion expression similar to that observed in the normal term placentae. All monoclonal antibodies stained a sparse spindle cell population seen underlying the amnion and chorionic epithelium.

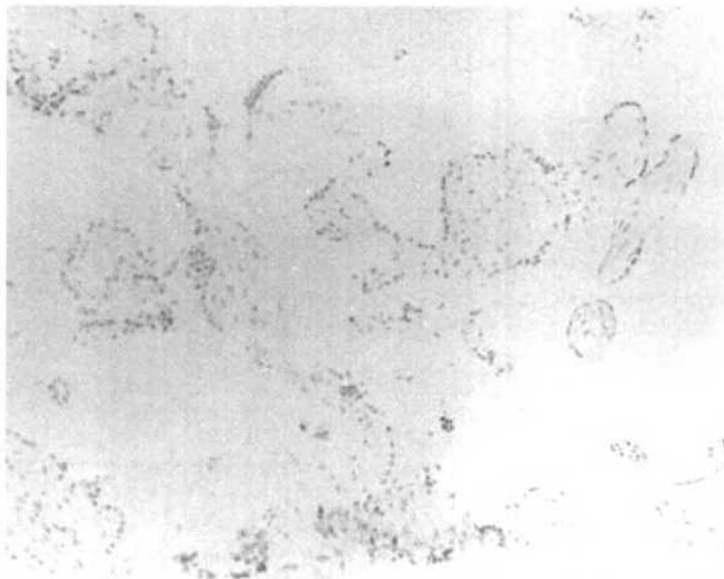


FIGURE 3. A term placenta shows a sparse stromal cell population stained with DA 6·231. Normal placenta. Immunoperoxidase.

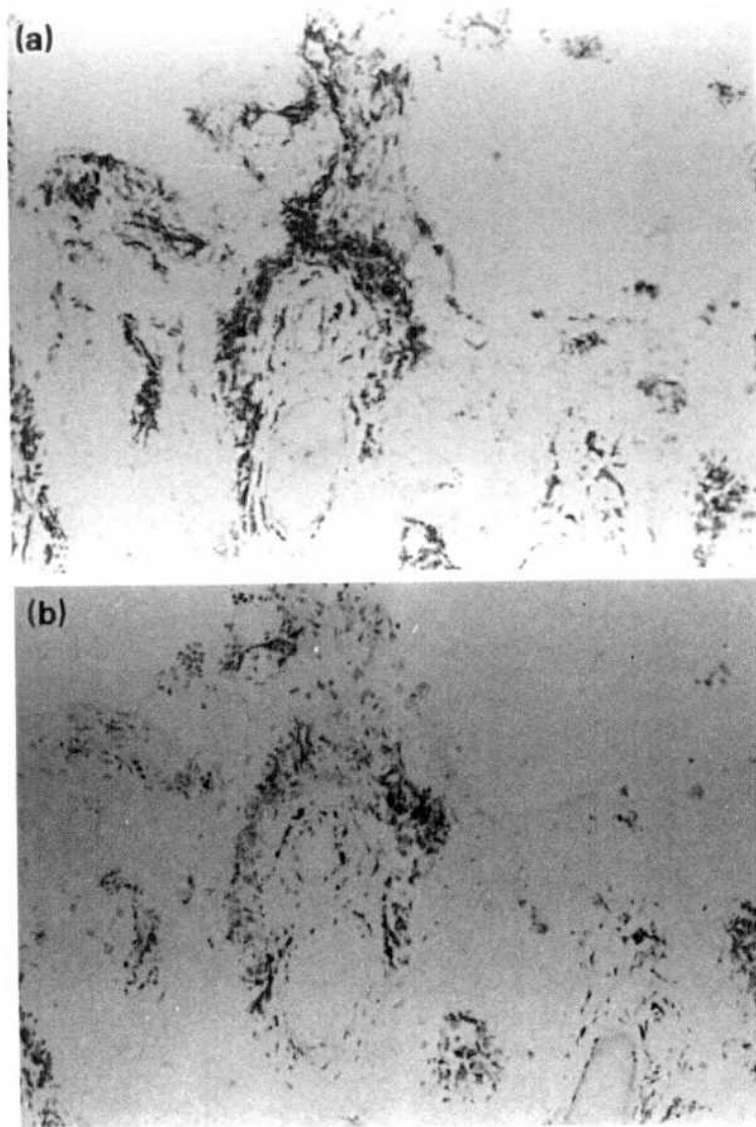


FIGURE 4. There is strong staining of stromal cells in chorionic villi of a PG placenta with monoclonal antibodies (a) DA 6-231 (b) B7/21. Placenta pemphigoid gestationis. Immunoperoxidase.

In all of the placentae there was focal staining of the endothelium of the chorionic villi with DA 6-231, DA 6-164 and B7/21 but not Leu 10 nor Tu 22.

Control experiments

All control sections were negative. The tonsil sections showed the previously described patterns of MHC Class II molecule expression.

DISCUSSION

Pemphigoid gestationis is an autoimmune skin disease associated with pregnancy in which circulating autoantibodies are directed against a BMZ antigen shared between the cutaneous and placental basement membranes.² It has been suggested that the autoimmune response is triggered by the exposure to the immune system of the antigen in the placenta.

We have shown that in the placentae from patients with PG, antibodies are deposited in the basement membranes of the placenta and at this site initiate complement fixation. By using monoclonal antibodies specific for IgG subclasses we identified IgG₁, as the major antibody in these placentae. Kelly *et al.*⁶ have previously shown that the main autoantibody in PG is of the IgG₁ subclass. It would appear that there is an immunological attack occurring in the placentae of patients with PG. There is clinical evidence of impaired placental function and hence foetal prognosis in PG.⁸ Holmes and Black⁸ suggested that this may be due to such an immunological attack on the placenta.

Patients with other forms of autoimmune subepidermal blistering diseases rarely become pregnant. We have been fortunate to study two patients with linear IgA disease during their pregnancies. The target antigen in linear IgA disease differs from the 180 kDa pemphigoid gestationis antigen (PGA).³ It is a 285 kDa molecule which on immunoelectron microscopy and split skin studies is found at a different site from either bullous pemphigoid antigen or PGA.⁹ Nevertheless we have shown that patients with linear IgA disease also exhibit immune deposits of IgA and C₃ in their placentae, compatible with an immunological attack on the placental BMZ. Placental function and foetal prognosis data in linear IgA disease are not available to compare with the PG data.

There are several other autoimmune diseases in which circulating tissue specific antibodies cause tissue damage.¹⁰ Recently, aberrant expression of the MHC Class II molecules by target organ cells has been demonstrated.¹¹⁻¹³ It has been suggested that these cells may then act as antigen presenting cells during dual recognition of antigen and MHC molecules by helper T lymphocytes thereby triggering the autoimmune response.^{11,12}

Although there is marked polymorphism in the MHC II region, monoclonal antibodies have been produced which recognize 'framework' determinants on the different subregion products.⁵ These antibodies have been used to study the expression of the MHC Class II subregion products in tissues. It has been shown by these methods that in several tissues, including placenta, DP is expressed before DR which is expressed before DQ.^{14,15} In our study the normal placenta also exhibited this progression of MHC II subregion product expression. The placenta at 12-16 weeks showed antibody binding patterns compatible with the expression of DP, at 22-26 weeks binding patterns compatible with the expression of DP + DR and at term all three subregion products were expressed. These findings confirm the previous report of Edwards *et al.*¹⁵

However, we found that in the term placentae from patients with PG there was greatly increased expression of MHC Class II subregion products by stromal cells and focal expression in the trophoblast. These results are similar to those obtained using a different panel of monoclonal antibodies.¹⁶ In both studies the population of cells showing MHC Class II expression was less extensively or consistently found. These two studies, therefore, suggest that as in other autoimmune diseases there is aberrant expression of MHC Class II molecules in PG.

Although immune complexes are found in these placentae, the expression of MHC Class II molecules is unlikely to be a secondary phenomenon because there are placental immune complexes in the cases of linear IgA disease, but aberrant MHC Class II expression is not a feature of these placentae.

By analogy with other autoimmune diseases, we suggest that this phenomenon is important in the initiation of the autoimmune response. However, several features of PG suggest that the mechanism of immune recognition may be unique to PG. We have shown that there is incoordinate aberrant expression of the MHC Class II subregion products in the placental stroma. This is seen in other cell types including those of the immune system but Gonwa *et al.*¹⁷ have analysed functional difference associated with incoordinate expression of MHC Class II subregions. They showed that cells showing incomplete MHC Class II expression functioned much less well as antigen presenting cells but were fully competent at evoking allogeneic responses in mixed leukocyte cultures. The placental cells which show aberrant expression of MHC II molecules would, therefore, seem to be poor antigen presenting cells. These cells, however, would be able to evoke an allogeneic response between the maternal lymphocytes and the paternal MHC molecules expressed on the foetal components of the placental stroma. Allogeneic mixed lymphocyte reactions can substitute for T lymphocyte helper function in initiating immune responses.¹⁸

Previous immunogenetic studies also suggest that the placental cells in PG do not act as antigen presenting cells. Co-operation between T lymphocytes and antigen presenting cells exhibits MHC restriction.¹⁹ Thus, antigen is only recognized by helper T cells if it is presented to them by cells expressing MHC Class II molecules identical to themselves. Holmes *et al.*²⁰ have described cases of PG in which second or subsequent pregnancies have been unaffected. They have shown that in some cases this occurs when the mother and foetus are fully compatible at the DR locus, but the autoimmune disease in these patients was seen when the mother and foetus showed allogeneic differences at MHC Class II.

Patients with PG also show positive evidence of allogeneic responses. Anti paternal MHC responses have been found in 85% of PG patients compared to 25% of normal pregnancies.²¹

Based on the above data our hypothesis is that in PG aberrant expression of MHC Class II subregion evokes local allogeneic responses in the placenta consequently triggering an autoimmune response against placental basement membrane antigens. This model would explain the immunogenetic data on PG²⁰ and is further supported by the demonstration of allogeneic responses in PG.²¹ Thus the mechanism of initiation of the immune response makes PG unique among organ specific autoimmune diseases.

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An investigation of circulating and *in situ* lymphocyte subsets and Langerhans cells in the skin and cervix of patients with chronic renal failure

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SUMMARY

This study was designed to detect possible changes in the immunocytology of the human immune system in the skin, cervix and peripheral blood of patients with chronic renal failure (CRF) treated by conservative methods, haemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD). In the skin, Langerhans cell numbers were reduced in CRF, CAPD and HD patients but in the cervix, Langerhans cells were reduced only in the CRF patients. There was a preponderance of T suppressor lymphocytes compared with T helper lymphocytes in the epidermis in the CRF and the CAPD groups. The presence of natural killer cells in the epidermis of the renal groups compared to controls was significant in the CRF and HD patients while the presence of T suppressor lymphocytes in the epidermis compared to controls was significant only in the CAPD patients. In the dermis, there was a mixed cellular infiltrate of T helper and T suppressor lymphocytes but with no subset attaining significance. The dermal infiltrate of T helper lymphocytes in all three groups of renal patients was significantly reduced compared to controls. In CRF patients, peripheral blood pan T cells, T helper and T suppressor lymphocytes and B lymphocytes were reduced, while T suppressor lymphocytes were reduced in both HD and CAPD patients, compared to controls. Though the results confirm alteration of the immunocytology of the skin, cervix and peripheral blood, we could not relate them to a clinical finding.

Uraemia is associated with impairment of immune function¹ and patients with chronic renal failure are more susceptible to infection.^{2,3} Persistent cutaneous infection such as with human papilloma virus (HPV) can be a serious problem and this virus is also associated with cervical neoplasia.^{4,5} Thus, it is of interest to know what influence uraemia has on the immune function of the skin and cervix of such patients and whether this is altered by different forms of dialysis.

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Previous investigators have assessed lymphocyte subsets in the peripheral blood of patients with chronic renal failure (CRF) treated by conservative methods, by continuous ambulatory peritoneal dialysis (CAPD) and by haemodialysis (HD) with conflicting results.⁶⁻⁸ There is no reported study characterizing the immunocytology of the skin and cervix of patients with chronic renal failure and no study has reported the prevalence of HPV in skin or cervix of such patients. We have, therefore, taken punch biopsies of the skin and cervix from patients with chronic renal failure and, using monoclonal antibodies, assessed the immunophenotype of the tissue obtained. Additionally lymphocyte subsets in the peripheral blood were enumerated.

METHODS

Subjects

Renal failure patients aged between 30 and 65 years were selected for the study. Eligibility for the CAPD and HD groups was treatment for a period of more than 6 months to 5 years. All patients with chronic renal failure treated by conservative means had a serum creatinine less than 500 $\mu\text{mol/l}$. Patients with multisystem autoimmune disease or who had a rejected a kidney transplant were excluded. Ethical Committee approval and informed consent was obtained from the patients prior to skin or cervical punch biopsy.

The patient groups investigated consisted of 15 with CRF (six males, nine females) aged between 33 and 61 years (mean 50 years); nine HD patients (six males, three females) who ranged in age from 31 to 60 years (mean 51 years) and 15 patients receiving CAPD (nine males, six females) aged from 33 to 60 years (mean 50 years). CAPD patients underwent 2L exchanges

TABLE 1. Immunohistological data for untreated chronic renal failure patients

Patient	Age	Diagnosis	Epidermal cell staining*					Dermal cell staining†		
			CD1	CD3	CD8	CD4	L7	CD3	CD8	CD4
001	39	Alport syndrome	11	2	5	—	—	++++	+++	+
002	42	Alport syndrome	21	1	1	—	3	+++	+++	+
003	51	Bilateral hydronephrosis	30	—	—	—	1	+++	+++	+
004	48	Chronic pyelonephritis	19	—	—	—	1	+++	+	+++
005	54	Hypertension	19	5	7	9	3	+++	++	++
006	60	Hypertension	20	—	—	—	—	++	++	++
007	61	Hypertension	25	—	—	—	—	+++	++	++
008	61	Hypertension	11	—	—	—	3	++	++	++
009	33	Membranous nephropathy	35	—	—	—	—	++	+++	+
010	52	Membranous nephropathy	17	—	—	—	—	++	++	++
011	44	Nephrotic syndrome	20	—	—	—	—	+++	++	++
012	61	Obstructive nephropathy	32	—	—	—	12	++	+++	+
013	46	Polycystic kidneys	12	5	3	—	—	+++	+	+++
014	54	Polycystic kidneys	16	1	1	—	—	++	+++	+
015	45	Polycystic kidneys	20	1	1	—	1	+++	+++	+

* Epidermal cell staining per 200 basal cells.

† Dermal cell staining: + = few cells, +++ = many cells.

TABLE 2. Immunohistological data for HD patients

Patient	Age	Diagnosis	Epidermal cell staining*					Dermal cell staining†		
			CD1	CD3	CD8	CD4	L7	CD3	CD8	CD4
016	60	Amyloidosis	13	5	3	2	10	++	+	+++
017	36	Chronic glomerulonephritis	29	—	—	—	—	+++	++	++
018	11	Chronic pyelonephritis	11	—	—	—	17	++	+++	+
019	54	Diabetic nephropathy	12	—	—	—	4	++	+	+++
020	31	Hypertension	8	—	—	—	—	+++	+	+++
021	53	Hypertension	16	—	—	—	—	++	++	++
022	57	Hypertension	6	—	—	—	4	+++	++	++
023	58	Hypertension	29	13	6	14	5	+++	++	++
024	49	Reflux nephropathy	16	—	—	—	—	++	++	++

* Epidermal cell staining per 200 basal cells.

† Dermal cell staining: + = few cells, +++ = many cells.

TABLE 3. Immunohistological data for CAPD patients

Patient	Age	Diagnosis	Epidermal cell staining*					Dermal cell staining†		
			CD1	CD3	CD8	CD4	L7	CD3	CD8	CD4
025	50	Analgesic nephropathy	17	8	1	—	—	+++	+++	+
026	48	Chronic glomerulonephritis	16	—	—	—	—	++	+	+++
027	52	Chronic glomerulonephritis	29	13	8	—	—	++++	+	+++
028	48	Chronic pyelonephritis	20	5	7	—	14	+++	+	+++
029	46	Diabetic nephropathy	30	4	7	—	2	+++	+	+++
030	48	Diabetic nephropathy	10	—	—	—	—	+++	+++	+
031	54	Hypertension	30	—	—	—	—	+++	+++	+
032	60	Hypertension	20	3	3	—	3	++	+++	+
033	33	Obstructive uropathy	27	5	3	4	—	+++	+++	+
034	56	Obstructive uropathy	22	3	3	—	2	++	++	++
035	45	Polycystic kidneys	20	12	5	7	4	+++	+	+++
036	52	Polycystic kidneys	13	—	—	—	—	++	+	+++
037	54	Polycystic kidneys	9	—	—	—	—	+++	+++	+
038	58	Polycystic kidneys	15	3	4	—	—	++	+++	+
039	48	Reflux nephropathy	24	4	3	—	—	+++	+++	+

* Epidermal cell staining per 200 basal cells.

† Dermal cell staining: + = few cells, +++ = many cells.

four times a day, while the HD group were dialysed three times a week for 4 h. Each patient's diagnosis is listed in the appropriate Table (1-3). Antihypertensive therapy in those patients receiving treatment consisted of varying combinations of diuretics, alpha and beta-adreno-receptor blocking agents and calcium channel blockers. Diabetic therapy was either diet or insulin injections.

The three control groups excluded patients who were diabetic, hypertensive or were taking any medication. Blood samples were obtained from 12 subjects with known normal renal function (five male, seven female) whose ages ranged from 26 to 76 years (mean 49 years). For control skin biopsies, normal skin was obtained from the periphery of non-inflamed cutaneous lesions excised from seven dermatology out-patients having scalpel excision. Biopsies of normal cervix were obtained from patients undergoing hysterectomy for reasons other than cervical disease or malignancy and from patients undergoing laparoscopic sterilization. All had negative smears and subsequent normal histology. The age range of controls was from 23 to 75 years (mean 44.2 years). Five patients were postmenopausal. All specimens were taken from the transformation zone, the area of the cervix in which neoplastic change originates.

Blood samples in the HD patients were taken prior to haemodialysis, immediately before systemic heparinization and, in CAPD patients, immediately before the next exchange. The following analyses were performed on the blood: full blood count, urea, electrolytes and creatinine and lymphocyte subtyping.

In those with renal disease, a 6 mm punch biopsy (Stiefel Laboratories, England) of skin was removed from the left arm of each individual under local anaesthesia (1% plain lignocaine). The female patients had a cervical smear, colposcopy and colposcopically directed punch biopsy of the cervix taken with Leech Wilkinson forceps.

TABLE 4. Monoclonal antibody specificities used to identify cell membrane antigens in the peripheral blood, skin and cervix

Monoclonal antibody	Cell recognized	Source
(a) Peripheral Blood*		
CD3	Pan T cells	Unipath
CD4	Helper T lymphocytes	Dako
CD8	Suppressor T lymphocytes	Royal Free Hospital
CD20	B lymphocytes	Coulter
Leu 7	Natural killer cells	Becton-Dickinson
(b) Skin and Cervix†		
CD1	Langerhans cells	Sera
CD3	Pan T cells	Scottish Antibody Production Unit
CD4	Helper T Lymphocytes	Unipath
CD8	Suppressor T lymphocytes	Scottish Antibody Production Unit
Leu 7	Natural killer cells	Becton-Dickinson
Leu 12	B lymphocytes	Becton-Dickinson

* The monoclonal antibodies were diluted in phosphate-buffered saline (1:10).

† The monoclonal antibodies were all diluted in tris buffer (1:100) except CD3 (1:80) and CD8 (1:50).

Immunohistological studies

Biopsies were snap-frozen in liquid nitrogen and stored at -70°C until cutting (cryostat sections were fixed in acetone prior to staining).

Mouse hybridoma monoclonal antibodies with specificities summarized in Table 4 were used for immunophenotyping. Endogenous immunoperoxidase was blocked with 7% hydrogen peroxide in tris buffer (0.1 M). The cryostat sections were incubated with monoclonal antibodies, washed in tris saline, and labelled with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) diluted 1:20 (1:1, normal human serum-tris buffer).

Skin and cervical sections were then reacted with diaminobenzidine and hydrogen peroxide, counterstained with Mayers haematoxylin, differentiated and blued. Skin sections were then counterstained with Azure B to differentiate melanin from diaminobenzidine positive staining, then dehydrated, cleared and mounted in Pertex medium. The alkaline phosphatase anti-alkaline phosphatase method⁹ was used for detecting natural killer cells in the skin. Simultaneous staining for pan T cells and the various subsets was not carried out.

Immunohistological assessment of skin and cervical biopsies

Skin. The epidermal cell number was evaluated as the number of positive staining cells per 200 basal cells for the antigen detected by the appropriate antibody. For the dermal papillary infiltrate, staining reactions for CD3 were scored on a semi-quantitative scale from 5+ to — (many cells to no cells stained). CD8 and CD4 were expressed as a percentage of CD3; 4+ to — (100% to 0%) (Tables 1–3, 5).

Skin recordings were evaluated by two independent assessors (at total magnification $\times 100$, $\times 250$), five fields were surveyed; the patient's groupings were not known to either.

Cervix. In the cervical epithelium, Langerhans cells were counted per 100 basal cells. The CD4 and CD8 fractions of the infiltrate were scored as a percentage of the pan T cells: 4+ to — (100% to 0%). In the stroma, the total infiltrate, CD3 was screened in a semiquantitative basis, 5+ to negative. CD8 and CD4 subsets were expressed as a percentage as in the epithelium. The Leu 12 and Leu 7 infiltrate were scored on the same basis as the pan T infiltrate (Tables 6, 7).

TABLE 5. Immunohistological data for control skin biopsies

Patient	Epidermal cell staining*				L7	Dermal cell staining†		
	CD1	CD3	CD8	CD4		CD3	CD8	CD4
040	25	—	—	—	—	+++	+	+++
041	35	—	—	—	—	+++	+	+++
042	31	—	—	—	—	+++	+	+++
043	32	—	—	—	—	++	+	+++
044	31	—	—	—	—	++	+	+++
045	25	—	—	—	—	+++	+	+++
046	34	—	—	—	—	+++	+	+++

* Epidermal cell staining per 200 basal cells.

† Dermal cell staining: + = few cells, +++ = many cells.

TABLE 6. Summary of immunohistological data from cervical biopsies of chronic renal failure patients

Patient	Epithelial cell staining			Stromal cell staining			L12	L7
	CD1	CD4	CD8	CD3	CD4	CD8		
004	4	—	++++	+++	+++	+	—	—
005	2	—	++++	++	+	+++	+	+
006	5	—	++++	+++	+++	+	—	—
008	8	—	++++	++	+++	+	—	—
011	8	+	+++	++++	+++	+	—	—
013	3	—	++++	++++	+++	+	++	—
014	3	+	+++	+++	++++	—	—	+
018	4	—	++++	++	+	+++	—	—
024	8	—	++++	++	+++	+	—	—
026	12	—	++++	+++	+++	+	—	+
029	6	—	—	++	+++	+	—	—
036	11	—	++++	++++	+++	+	+	—
038	4	—	++++	++++	+++	+	+	—
039	4	—	—	++	+++	+	—	—

Quantitation of lymphocytes

All patients were venesected between 09.00 and 10.00 to minimize effects due to diurnal variation.

Heparinized blood samples were diluted in Hank's balanced salt solution without calcium and magnesium. Mononuclear cells were isolated by separating blood on a Ficoll-Hypaque gradient (Lymphoprep) and then washed twice for 10 min in phosphate-buffered saline (PBS). To 20 μ l of antibody (Table 4) was added 200 μ l of solution of PBS containing 1×10^6 cells and this was incubated for 30 min on ice. After washing twice in PBS, the cells were incubated for a further 30 min on ice with 100 μ l of 1 in 10 dilution of Dako rabbit anti-mouse immunoglobulin F(ab)₂ fragments in PBS conjugated to fluorescein isothiocyanate. Following two further washings in PBS, the cells were analysed through a FACS analyser (Becton-Dickinson Immunocytometry Systems, Mountain View, CA, U.S.A.). Lymphocytes were resolved from monocytes and contaminating granulocytes by correlation of wide angle light scatter and cell volume. Gates were then placed around the lymphocyte population to exclude all other cells. Fluorescent signals from 1×10^6 lymphocytes were then analysed for each antibody (Table 8).

TABLE 7. Mean Langerhans cells per 100 basal cells in biopsies from normal transformation zone of the cervix

	No. of subjects	Mean
Control	25	8.0 $\pm$ 3.1
CAPD	5	7.4 $\pm$ 3.8
Haemodialysis	2	6.0 $\pm$ 2.8
CAPD/Haemo	7	7.0 $\pm$ 3.2
Chronic renal failure	7	5.3 $\pm$ 2.7

TABLE 8. Lymphocytes and natural killer cells in the peripheral blood of chronic renal failure patients

Variable	CAPD	Haemodialysis	Untreated chronic renal failure	Controls	P value
Pan T	1126 ± 637	1013 ± 279	731 ± 226	1969 830	<0.001
T helper	741 ± 437	664 ± 154	462 ± 150	1205 ± 452	<0.001
T suppressor	428 ± 280	356 ± 135	282 ± 129	860 ± 454	<0.001
Natural killer cells	320 ± 215	433 ± 237	297 ± 201	709 ± 283	>0.05
B lymphocytes	147 ± 15	152 ± 115	81 ± 48	314 ± 146	<0.05

Results are expressed as the mean and one standard deviation.

Statistical methods

The data were expressed as means and 1 standard deviation ($\pm$ SD). Peripheral blood lymphocyte data were analysed using a one-way analysis of variance, with a follow-up Bonferroni multiple comparison procedure.¹⁰ Skin and cervical Langerhans cell numbers were assessed using an 'unpaired *t*-test' for comparison of each of the three groups (CRF, HD and CAPD) with the control. The frequency with which T suppressor and T helper lymphocytes occurred in the epidermis and dermis of each group was assessed by the Chi-squared or Fisher exact test where appropriate. The frequency of natural killer cells and T suppressor lymphocytes in the epidermis and T helper lymphocytes in the dermis of each renal group compared to controls was assessed by the Fisher exact test. Components of the cervical infiltrate between the renal failure groups and controls were analysed using a one-way analysis of variance.

RESULTS

Skin and cervical immunohistological studies

Skin. In the control skin biopsies the mean Langerhans cell number was 30.43 (SD 3.99). There was no epidermal T cell staining. In the dermal lymphocytic cell infiltrate T helper lymphocytes predominated (Table 5). Our results are similar to a previous investigation from this unit¹¹ on Langerhans cell numbers in normal human epidermis, in the upper limb of 26 patients, which used the same method for identification of these cells (standard immunoperoxidase technique) with findings of a mean 29 (SD 13.4).

Chronic Renal Failure (Table 1): in the 15 untreated CRF patients, the mean Langerhans cell number was 20.53 (SD 7.29). This was significantly fewer compared to controls ($P < 0.003$). Suppressor lymphocytes were present in the epidermis in six of the CRF patients and natural killer cells accompanied them in half the cases and were themselves present in another three patients. The presence of T suppressor lymphocytes compared to T helper lymphocytes in the epidermis was significant ($P < 0.05$) and the presence of natural killer cells in the epidermis

compared to controls was also significant ($P < 0.05$). In the dermal cell infiltrate, neither lymphocyte subset predominated; in comparison to the control the T helper lymphocytes' dominance in the dermal infiltrate was reduced ($P < 0.001$).

Haemodialysis (Table 2): the mean Langerhans cell number in the nine HD patients was 15.56 (SD 8.29); this was reduced compared to controls ($P < 0.001$). Suppressor and helper lymphocytes were present in two patients, along with natural killer cells in the epidermis, while natural killer cells were present in three other patients. Natural killer cells' presence in the epidermis compared to controls was significant ($P < 0.05$). No lymphocyte subset's presence was significant in the epidermis or the dermis. The T helper lymphocytes' dominance in the dermal infiltrate was significantly reduced compared to controls ($P < 0.02$).

CAPD (Table 3): the 15 CAPD patients had a mean Langerhans cell number of 20.13 (SD 6.92), significantly reduced compared to controls ($P < 0.002$). In 10 patients suppressor lymphocytes were present in the epidermis, with natural killer cells present in half of these. The presence of T suppressor lymphocytes in the epidermis compared to controls was significant ($P < 0.005$). T suppressor lymphocytes' presence in the epidermis compared with T helper lymphocytes was significant ($P < 0.005$). No significance was found for lymphocyte subsets in the dermis but in comparing the dominance of T helper lymphocytes to the controls there was a significant reduction ($P < 0.01$).

Cervix. Langerhans cells were reduced in the epithelium of CRF patients ($P < 0.02$) compared with controls but not in the HD and CAPD patients (Table 7). One-way analysis of variance showed no significant differences in the components of the infiltrate between normals and the three subgroups examined. T suppressor lymphocytes predominated in the epithelium and helpers in the stroma in all groups.

Gross findings in the skin and cervix. There was no evidence of human papilloma wart virus, cutaneous infection or cancer in the skin. Colposcopic examination revealed the presence of wart virus in one patient but no evidence of cervical-intraepithelial neoplasia (CIN).

Lymphocyte quantitation. The Bonferroni multiple comparisons procedure showed a significant reduction in the peripheral blood between controls and those with CRF for pan T lymphocytes, T helper and T suppressor lymphocytes ($P < 0.001$) and B lymphocytes ($P < 0.05$); and between controls and the T suppressor lymphocytes in HD ($P < 0.001$) and CAPD ($P < 0.02$) patients (Table 8).

DISCUSSION

Frequent infections¹ and a high incidence of neoplasia have been reported in patients undergoing long term intermittent dialysis.¹² Uraemic patients have decreased delayed hypersensitivity to common antigens;¹³ and skin homografts from this group have a longer survival compared to their survival in normal subjects.¹⁴

Langerhans cells are immunocompetent bone-marrow derived cells,¹⁵ involved in cell-mediated immune reactions¹⁶ particularly in antigen presentation¹⁷⁻¹⁹ and allograft rejection.²⁰ Langerhans cells are a consistent component of the skin and cervical epithelium as identified on light microscopy²¹⁻²³ and electron microscopy²⁴⁻²⁷ and by using various staining techniques.^{21,22,28,29} They act in both the skin and the cervix, as the peripheral outpost of the immune surveillance system. In the skin, Langerhans cell numbers are reduced with the use of

PUVA³⁰ and corticosteroids³⁰ and increased with melanocytotoxins.³¹ Cervical Langerhans cells are reduced where there is histological evidence of HPV infection alone;³²⁻³⁴ and where there is evidence of CIN with concomitant HPV infection.³⁴ In this investigation we have found reduced numbers of Langerhans cells in the cervix of non-dialysed chronic renal failure patients and normal numbers in the dialysed patients. The findings suggest that treatment of uraemia restores Langerhans cell numbers to within the normal range. This difference was not seen in the skin in this series.

An increased risk of neoplasia has been reported in renal transplant recipients.³⁵ Penn³⁶ found the incidence of neoplasia to increase with the length of time from transplantation. One group³⁷ calculated a 14-fold increased risk of cervical neoplasia in renal transplant recipients compared to age matched controls in their area. Abnormal cytology on routine smear examination was the commonest presentation. We, therefore, felt that investigating these groups prior to transplantation would be useful in terms of determining the prevalence of wart virus, CIN and neoplasia. Only one patient had clinical evidence of cervical HPV, which was confirmed on histological examination; none had neoplastic changes or CIN. Available evidence suggests T cell mechanisms predominate in the cervix, while B lymphocytes appear to have little importance in the normal or abnormal cervix.^{32,38,39} The predominant lymphocyte subtype in cervical epithelium is the cytotoxic/suppressor group, whereas in the stroma it is the helper T lymphocytes.³⁸⁻⁴⁰ Our findings are in agreement with this work.

In the skin of both dialysed and non-dialysed chronic renal failure patients, Langerhans cell numbers were significantly reduced compared to our controls and to those of a previous study.¹¹ This may be due to a reduced turnover or decreased migration to the skin of Langerhans cells. The significance of reduced skin Langerhans cells is uncertain, but in view of the proposed role of Langerhans cells it may be a partial explanation as to why skin homografts have increased survival in uraemic patients compared to their survival in normal subjects.¹⁴

There are several notable findings from the skin immunohistology results; the presence of suppressor lymphocytes and natural killer cells in the epidermis, alone or in combination, and the presence of occasional helper lymphocytes in the epidermis only if suppressor lymphocytes were present. In the epidermis of the renal groups compared to controls the presence of natural killer cells was significant in CRF ($P < 0.05$) and the HD ($P < 0.05$) patients while T suppressor lymphocyte presence was significant in the CAPD group ($P < 0.005$). Epidermal T suppressor lymphocyte presence compared to T helper lymphocytes was significant in the non-dialysed chronic renal failure patients ($P < 0.05$) and in the CAPD group ($P < 0.005$). In the dermis of all renal groups the numbers of T helper lymphocytes were significantly reduced compared to controls. The reason for such differences is unknown.

Analysis of T lymphocyte subtyping in peripheral blood has produced conflicting data. Giacchino *et al.*⁶ found that peripheral T cell subsets in CAPD patients showed no significant change from normal. Raska *et al.*⁸ found that in haemodialysis patients, T helper and suppressor cells were reduced in patients between the ages of 30 and 60 years, but only T helper cells were reduced in those over 60. Collart *et al.*⁷ found in comparing T lymphocyte subtypes in CAPD and haemodialysis patients that pan T and T helper cell numbers were not altered but that T suppressor numbers were reduced in both groups. Our findings are in agreement with those of Collart *et al.*,⁷ confirming that neither treatment modality CAPD or HD is able to restore peripheral blood T suppressor numbers to normal levels.

Overall, the findings suggest that in renal failure patients there is the presence and/or the reduction of various immunocompetent cells in the skin, cervix and peripheral blood. There was

no evidence of infection or skin neoplasia. Patients were asymptomatic for pruritus. Anti-hypertensive and diabetic therapy have not been reported to influence the immune system of the skin, cervix or the peripheral blood. We were unable to relate the immunohistological results to a clinical finding. However, we suggest that serial skin biopsies with immunohistological staining from a larger number of patients, from the onset of their presentation with renal failure and their follow through to either CAPD or HD, may shed further information as to whether the immunocytological findings are constant or vary with the duration of the disease and about their possible association with clinical findings.

In conclusion, although our study is limited by a small number of patients and controls we have shown that there is an alteration in the immune status of renal failure patients and we hope this will stimulate further studies.

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Release of prostacyclin from cultured human endothelial cells following prolonged exposure to histamine

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SUMMARY

Histamine is an important pro-inflammatory molecule mediating leukocyte margination, plasma extravasation and vasodilation, but its precise mode of action on vascular endothelium is unclear. We report that histamine is able to induce prolonged release of prostacyclin (PGI₂) from human endothelial cells via occupancy of the H₁ receptor, without an absolute requirement for the presence of histamine or synthesis of new enzyme protein to facilitate continued release of PGI₂.

Under basal conditions endothelial cells, cultured from either bovine aorta (BAEC) or human umbilical vein (HUVEC), release minimal amounts of prostacyclin (PGI₂). However, stimulation with a variety of agonists including interleukin-1 (IL-1),¹ the calcium ionophore A23187, thrombin² and histamine³ induces release of PGI₂. Although little is known about the specific mechanisms involved in the induction and regulation of PGI₂ synthesis within endothelial cells, there is now evidence to suggest that these events are mediated by at least two second messenger pathways. PGI₂ release from HUVEC incubated with the pro-inflammatory cytokine IL-1 α , is characterized by a delayed onset of 4-6 h but is then continuous up to at least 48 h, as long as the agonist is present.⁴ This is in direct contrast to previous data concerning the release of PGI₂ induced by agonists such as thrombin² or histamine,³ which indicated that these agonists induced an immediate release of PGI₂ from both BAEC and HUVEC which was maximal within 30 min, the endothelial cells then becoming refractory to repeated stimulation. There is however no data regarding the release of PGI₂ following prolonged exposure of endothelial cells to these agonists, as is required for the induction of PGI₂ release by IL-1 α . Histamine is an important pro-inflammatory molecule mediating leukocyte margination and plasma extravasation as well as vasodilatation, although its precise mode of action on endothelium is still unclear.

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METHODS

Materials

Culture medium and antibiotics were obtained from Gibco, Uxbridge, Middlesex, U.K. Histamine, cycloheximide, actinomycin D, tunicamycin, indomethacin, pyrilamine (maleate salt), dibutyryl cyclic adenosine monophosphate (cAMP), iso-butyl-methylxanthine (IBMX) and trifluoperazine were obtained from Sigma Chemical Co., U.K. Cimetidine was obtained from Aldrich Chemical Co., Gillingham, U.K. and collagenase from Worthington Biochemicals, U.K.

Endothelial cell culture

Human endothelial cells (HUVEC) were harvested from umbilical cord veins and cultured as previously described.⁵ For these experiments, the cells were seeded into 24 well cluster plates and cultured in complete medium consisting of medium 199 (M199) containing 100 u/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml amphotericin B, 2mM glutamine and supplemented with 20% pooled human serum. The cells used in these experiments were primary cultures and they were identified as endothelial cells by positive immunofluorescent staining for von Willebrand Factor protein.⁶

Histamine induced release of prostacyclin from endothelial cells

Confluent monolayers of endothelial cells were washed once with M199 alone. Each well of endothelial cells was then overlaid with 500 µl of M199 containing histamine at concentrations of 10^{-7} M to 10^{-3} M. The cells were incubated at 37°C for either 30 min or 24 h, after which time the supernatants were removed from an individual well and frozen at -20°C for subsequent radioimmunoassay of 6-keto-prostaglandin $F_{1\alpha}$ (6 keto-PG $_{1\alpha}$). At the end of the experiment, the endothelial cells were trypsinized (0.25% w/v trypsin in Hanks balanced salt solution (HBSS))

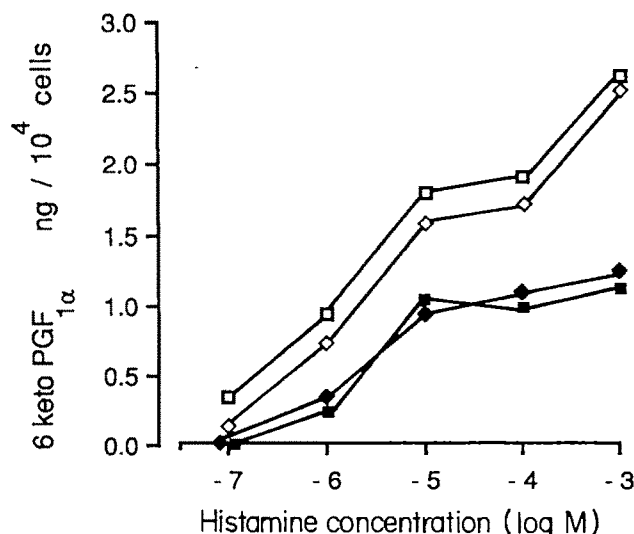


FIGURE 1. Dose dependent release of PGI $_2$ from cultured HUVEC following 30 min [■, ◆] and 24 h [□, ◇] incubation with histamine. The results of two separate experiments are shown.

and counted manually using an improved Neubauer Chamber (Weber, Lancing, Sussex, U.K.). Concentrations of 6 keto-PGF_{1α} were expressed as ng/10⁴ cells.

In order to determine the time course and whether the continuous presence of histamine was an absolute requirement for PGI₂ release, endothelial cells were exposed to either 10⁻⁵M or 10⁻⁴M histamine, and at different time points between 30 min and 24 h the supernatant was removed from an individual well of endothelial cells and replaced with a fresh aliquot of M199 alone. The incubations were continued for up to 24 h. Both aliquots of cell supernatant were assayed for 6 keto-PGF_{1α}.

The effect of specific antagonists on release of prostacyclin from endothelial cells

Confluent monolayers of endothelial cells were washed with M199 and overlaid with 500 μl of M199 containing the different antagonists. Following 30 min incubation at 37°C, histamine (10⁻⁵M final concentration) was added and the cells incubated for a further 30 min or 24 h at 37°C, at which time the supernatant was removed and frozen at -20°C for subsequent radioimmunoassay for 6 keto-PGF_{1α}.

Prostacyclin assay

PGI₂ release from endothelial cells was measured using a radioimmunoassay for 6 keto-PGF_{1α}, the stable hydrolysis product of prostacyclin, as previously described.⁷ The 6 keto-PGF_{1α} antiserum exhibited cross-reactivity of less than 1% with other major prostaglandins of the 1 and 2 series and the limit of sensitivity of the assay was 50 pg/ml. The cell supernatants were assayed without prior extraction and measurements made at two dilutions.

RESULTS

Histamine induced a concentration dependent release of PGI₂ between 10⁻⁶M and 10⁻³M, as shown in Fig. 1. Following 30 min incubation, release of PGI₂ was maximally induced by 10⁻⁵M

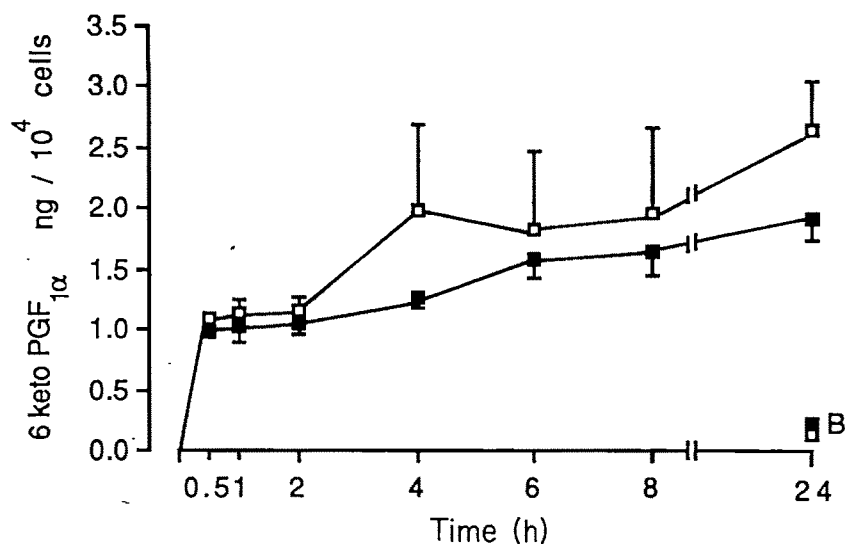


FIGURE 2. The time course of PGI₂ release from HUVEC following exposure to 10⁻⁵M [■] or 10⁻⁴M [□] histamine. 'B' is basal release of PGI₂ following 24 h incubation. The results represent the mean ± standard error of the mean (SEM). *n* = 4.

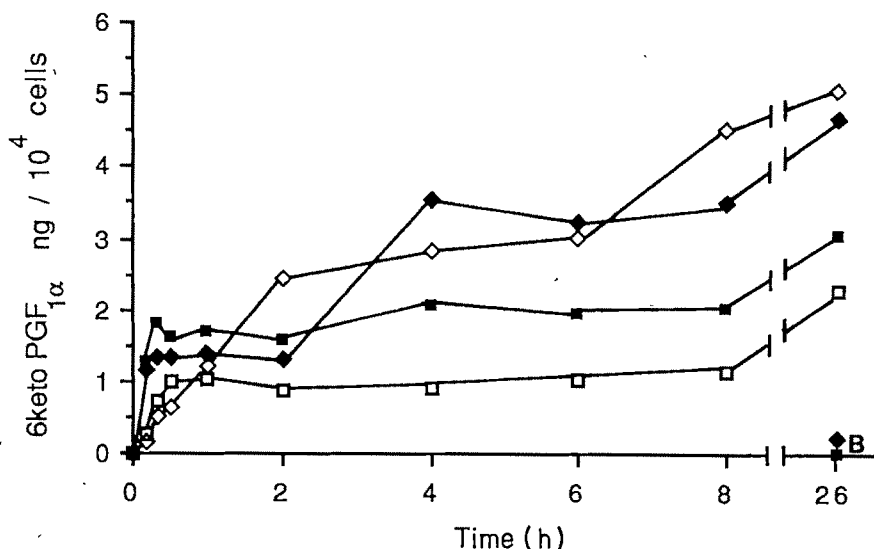


FIGURE 3. The time course of release of PGI₂ from HUVEC stimulated with 10^{-4} M histamine showing release of 6 keto-PGF_{1α} in the presence of the agonist [■, ◆] and following removal of the agonist [□, ◇]. 26B is the basal release following 26 h incubation. The results of two separate experiments are shown.

histamine. Continued incubation of the cells with histamine up to 24 h resulted in further release of PGI₂ which was maximal with 10^{-3} M histamine. At the end of the incubation period, the endothelial cell monolayer was intact and cell viability was greater than 95%, as evidenced by trypan blue exclusion.

Detailed examination of the time course of release of PGI₂ from HUVEC incubated with either 10^{-5} M or 10^{-4} M histamine indicated the release occurred by two mechanisms (Fig. 2). There was an initial rapid release of PGI₂ which occurred within 30 min of stimulation. This was followed by a lag phase of 2 h, after which time release of PGI₂ began to increase again up to 24 h when the incubations were terminated. Sustained gradual release of PGI₂ was observed in HUVEC incubated with both 10^{-4} M and 10^{-5} M histamine, and in HUVEC incubated with 10^{-4} M but not 10^{-5} M histamine, there was substantial release of PGI₂ between 2 and 4 h. The prolonged release of PGI₂ from HUVEC exposed to 10^{-4} M histamine did not exhibit an absolute requirement for the presence of histamine as release of PGI₂ continued after removal of the agonist (Fig. 3).

Prior incubation of the HUVEC with either 10^{-6} M or 10^{-5} M pyrilamine (H₁ receptor antagonist) inhibited the initial phase of histamine induced PGI₂ release and partially inhibited secondary release (Table 1). In contrast, prior incubation of HUVEC with either 10^{-6} M or 10^{-5} M cimetidine (H₂ receptor antagonist) did not inhibit either the initial phase or subsequent release of PGI₂. Inhibition of histamine induced release of PGI₂ from HUVEC incubated with pyrilamine was not prevented by co-incubation with cimetidine.

In an attempt to determine the metabolic events mediating endothelial cell PGI₂ release, HUVEC were incubated with actinomycin D (RNA transcription inhibitor), cycloheximide (protein translation inhibitor) and tunicamycin (post translation glycosylation inhibitor). In preliminary experiments, the endothelial cells were incubated with varying concentrations of lone antagonists for 24 h to assess their direct effect on endothelial cell morphology and PGI₂

TABLE 1. The effect of H₁ (pyrilamine) and H₂ (cimetidine) receptor antagonists on the histamine induced release of PGI₂ from cultured human endothelial cells

Receptor antagonists		6keto-PGF _{1α} % max. release ± SEM	
		30 min	24 h
Pyrilamine	10 ⁻⁵ M	6.49 ± 3.42	39.42 ± 12.21
	10 ⁻⁶ M	0	29.45 ± 10.58
Cimetidine	10 ⁻⁵ M	81.10 ± 10.00	127.66 ± 15.51
	10 ⁻⁶ M	111.20 ± 2.41	107.06 ± 11.64
Pyrilamine + Cimetidine	10 ⁻⁵ M	2.47 ± 2.47	43.48 ± 17.40

Confluent monolayers of human endothelial cells were pre-incubated with the antagonists for 30 min prior to the addition of 10⁻⁵M histamine. The incubation was then continued for either 30 min or 24 h.

The results are the mean ± SEM of two or three experiments and are expressed as a % of 6 keto-PGF_{1α} released in the presence of 10⁻⁵M histamine alone.

TABLE 2. The effect of metabolic antagonists on histamine induced release of PGI₂ from cultured human endothelial cells

Metabolic antagonist		6keto-PGF _{1α} % max. release ± range
Cycloheximide	180 nM	108.90 ± 4.11
	18 nM	95.02 ± 0.84
Actinomycin D	8 nM	120.38 ± 18.77
	800 pM	110.46 ± 5.76
Tunicamycin	6 μM	99.23 ± 9.59
	600 nM	108.82 ± 6.25
Cholchicine	13 nM	118.95 ± 16.35
	1 nM	97.44 ± 0.28

Confluent monolayers of human endothelial cells were pre-incubated with the antagonists for 30 min prior to the addition of 10⁻⁵M histamine. Incubation was then continued for 24 h.

The results are the mean ± range of two experiments and are expressed as a % of 6 keto-PGF_{1α} released in the presence of 10⁻⁵M histamine alone.

TABLE 3. The effect of functional antagonists on histamine induced release of PGI₂ from cultured human endothelial cells

Functional antagonists		6 keto-PGF _{1α} % max. release ± range
Trifluoperazine	104 μM	101.41 ± 15.86
	10 μM	107.58 ± 0.63
Indomethacin	10 μM	5.24 ± 1.75
	1 μM	4.58 ± 0.46
Dibutyl cAMP	10 μM	90.73 ± 3.17
	1 μM	104.90 ± 3.53
IBMX	1 mM	122.36 ± 0.18
	100 μM	93.67 ± 9.38

Confluent monolayers of human endothelial cells were pre-incubated with the antagonists for 30 min prior to the addition of 10⁻⁵M histamine. The incubation was then continued for 24 h.

The results are the mean ± range of two experiments and are expressed as a % of 6 keto-PGF_{1α} release in the presence of 10⁻⁵M histamine alone.

release. Only those concentrations shown to induce neither morphological change, as assessed by phase contrast microscopy, nor direct stimulation of PGI₂ release, were used for these experiments. Histamine induced release of PGI₂ from HUVEC was unaffected by co-incubation with these antagonists indicating that new protein synthesis was not involved (Table 2).

PGI₂ release has been associated with increased release of cAMP, but exogenous cAMP and the phosphodiesterase inhibitor IBMX did not inhibit histamine induced release of PGI₂. Regulation of cytosolic calcium levels has been shown to be mediated by binding of calcium to calmodulin but trifluoperazine, which inhibits this binding, did not inhibit PGI₂ release. The only antagonist to exert an inhibitory effect was indomethacin. All stages of PGI₂ release from HUVEC was completely inhibited by 10 μM (Table 3).

DISCUSSION

Histamine released from basophils, mast cells or nerve fibres is an important mediator of not only cutaneous, but also systemic inflammatory and allergic responses.⁸ Although the vasoactive properties of histamine have been extensively demonstrated, its mode of action is poorly understood.

We report here that histamine is able to induce prolonged release of PGI₂ from HUVEC. The initial rapid release of PGI₂ is maximal within 30 min and is inhibited by the H₁ receptor antagonist pyrilamine but not by the H₂ receptor antagonist cimetidine. These data are in agreement with previous results.⁹ However, we have found that following a lag phase, release of PGI₂ began to increase again and continued to do so up to at least 24 h. In human dermal microvascular endothelial cells, the slow protracted release of prostaglandin E₂, induced by IL-1, is inhibited by cycloheximide, indicating that new protein synthesis is required.¹⁰ However, the

cellular requirements mediating the prolonged release of PGI₂ from HUVEC incubated with histamine are unclear, as a variety of metabolic and functional inhibitors, at concentrations reported to be efficacious in a variety of cell types, failed to inhibit histamine induced release of PGI₂.

Regulation of PGI₂ release from vascular endothelial cells is poorly understood. It has been reported that both the cyclo-oxygenase and prostaglandin synthetase enzymes are rapidly inactivated by peroxidase free radical generation.^{11,12} However, Haddock *et al.*¹³ have reported that recovery from tachyphylaxis to histamine is dependent on the availability of the histamine degradative enzymes diamine oxidase and methyltransferase and inhibited by the continued presence of histamine. We have shown that exposure of HUVEC to histamine induced prolonged release of PGI₂ which did not appear to exhibit an absolute requirement for the presence of the agonist. These apparently conflicting results may be accounted for by the existence of a third receptor for histamine and/or its metabolites such as methylhistamine, or alternatively that histamine metabolites are able to bind to H₁ receptors, so maintaining receptor occupancy and second messenger activation. The existence of a third (H₃) histamine receptor, which inhibits histamine synthesis and release, has recently been described in both cerebral cortex¹⁴ and perivascular nerve terminals.¹⁵ The recent development of specific H₃ agonists and antagonists¹⁶ will enable their existence on endothelium to be determined.

Release of PGI₂ from endothelial cells is associated with accumulation of both cAMP¹⁷ and cGMP,¹⁸ although a causal relationship between these molecules and PGI₂ release has not been unequivocally established. In sympathetic ganglia, histamine H₁ and H₂ receptors have been linked to changes in cGMP and cAMP respectively¹⁹ and more recently histamine has been shown to induce inositol phosphate accumulation via H₁ receptors in HUVEC.²⁰ The results reported here would suggest that cAMP and the adenylate cyclase second messenger system play only a minor role in the long-term regulation of PGI₂ release from HUVEC and that histamine induces release via the inositol phosphate second messenger system.

Berridge²¹ has suggested that stimulation of cell functions mediated by the inositol phosphate second messenger systems results in considerable overproduction of the second messenger before the diacylglycerol/C-kinase negative feedback loop becomes effective in bringing the cellular responses under control. The observed lag phase between the first and second stages of PGI₂ release may represent a period after which there is a controlled activation of the second messenger system, mobilization of arachidonic acid and gradual release of PGI₂. Sustained influx of calcium ions has been reported in association with continued H₁ receptor occupancy²² which may be sufficient to maintain activation of the second messenger system.

Although histamine is known to promote vasodilatation and plasma extravasation, the underlying mechanisms are poorly understood. The evidence supporting the existence of H₁ and H₂ receptors on endothelium is limited. Labelling of vascular endothelium in mouse diaphragm with histamine-ferritin conjugates indicated preferential binding on the luminal plasmalemma, especially in regions rich in filaments and near junctions between endothelial cells. Histamine binding was highest in post capillary venules where there was a preponderance of H₂ receptors. A similar situation existed in the capillaries, but the total number of receptors was less than half that in the venules. Arterioles were endowed with equal numbers of H₁ and H₂ receptors but the total number of receptors was ten-fold less than in the venules.²³ These results suggested that the opening of endothelial cell junctions and plasma extravasation were mediated primarily via H₂ receptor occupancy, but our results suggest that histaminergic effects on endothelium are mediated primarily via H₁ receptor occupancy. Albumin diffusion through intercellular gap junctions in HUVEC and BAEC monolayers is mediated by H₁ receptors.^{22,24}

In HUVEC this process is associated with sustained influx of calcium ions and reduced F-actin content.²² Similar results have been obtained in mice, where histamine induced capillary permeability was mediated solely via H₁ receptors.²⁵

The various vascular beds respond differently to histamine and this may be due to the types of histamine receptors they possess. Microvascular endothelium releases more prostaglandin E₂ than PGI₂,²⁶ but it is not known at present which prostaglandin is the major arachidonic acid metabolite in resistance vessels.

The complexities of histamine/endothelial cell interactions are further highlighted by the variable *in vivo* response to antihistamines in inflammatory conditions where histamine has been implicated such as the chronic and physical urticarias. The results reported here may offer an explanation for the persistent erythema observed in these conditions and may explain the lack of benefit of combined H₁ and H₂ receptor blockers in treatment of some chronic and physical urticarias. They may also provide a link between the increased histamine and prostaglandin levels observed in association with UV-induced erythema.^{27,28}

In summary, histamine induces a biphasic release of PGI₂ from cultured human endothelial cells but the mechanisms facilitating this release remain to be elucidated. These results underline the complex mechanisms regulating both endothelial cell release of prostaglandins and response to histaminergic stimulus.

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UVA sunbeds: tanning, photoprotection, acute adverse effects and immunological changes

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SUMMARY

The effects on 31 normal subjects following exposure to sunbeds containing UVA lamps with minimal UVB emission have been compared in a double-blind study with the effects on nine control subjects of a similar exposure course three times weekly for 4 weeks to sunbeds emitting visible light. On previously untanned areas, all those subjects on active treatment developed a mild tan; in tanned areas they all developed a moderate tan, while all control subjects developed a minimal to mild tan. The mean protection factor against later UVB-induced erythema was 3.2 ± 0.3 after the active course and 1.6 ± 0.2 among the controls. Significantly more frequent adverse cutaneous effects for active subjects were pruritus, erythema, freckling, burning sensation, dryness and polymorphic light eruption. Cutaneous Langerhans cell numbers, and blood CD3⁺ (pan T-cell) and CD4⁺ (helper T-cell) lymphocyte subsets were reduced in both active and control groups. CD8⁺ (cytotoxic/suppressor T-cell) counts were similarly but not significantly reduced in both groups. *Pityrosporum* yeast counts were significantly reduced in both groups. The changes found in both groups seem attributable to small amounts of UVB emission from both active and control lamps.

The use of cosmetic ultraviolet (UV) irradiation skin tanning units, especially lie-on UVA (315-380 nm) sunbeds, has rapidly increased in recent years,¹⁻³ mainly because of the belief that they tan the skin without burning or other significant adverse effect. However, short-term effects of such irradiation have in fact included erythema, pruritus, polymorphic light eruption, photosensitization reactions,⁴ pyrimidine dimer formation,⁵ exacerbation of light-sensitive dermatoses such as lupus erythematosus⁶ and impairment of immune function,⁷ whereas prolonged UVA exposure has caused animal^{8,9} and human¹⁰ dermal degenerative effects, and, at least in animals, accelerated development of skin cancer.^{11,12} Corneal and lens damage may also occur.¹³ Furthermore, only a third of subjects in one study received significant tanning after a sunbed treatment course and tolerance induced against UVB erythema was only moderate.¹ Recent reports of possible pityriasis versicolor,^{14,15} atypical lentiginos,¹⁶ and malignant

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melanoma^{17,18} following sunbed use have now made the safety of these devices even more uncertain.

Previous sunbed lamps have emitted significant UVB (280–315 nm) radiation, which has been suggested as the cause of adverse changes in the skin. We have investigated the effects of a course of exposure to sunbeds that contain new UVA lamps that emit much smaller amounts of UVB.

METHODS

Subjects

A double-blind controlled trial was undertaken during the winter months from January to March on 40 untanned white adult volunteers of ages 16–54 years and of variable burning and tanning skin types (Table 1). Subjects suffering severe photosensitivity, lupus erythematosus, skin tumours, eye cataracts or taking or applying known photosensitizing agents were excluded. Twenty volunteers, the maximum possible in the working hours available for irradiation, were allocated randomly for UVA (6 female, 5 male) and controls (5 female, 4 male) for the first 4 weeks of the study. A further 20 (14 female, 6 male) had UVA exposure for the final 4 weeks of the study.

Sunbeds

Two commercial sunbeds were used together for simultaneous irradiation of active and control subjects over the first 4-week exposure course, and for simultaneous irradiation of active subjects during the second 4-week exposure course. They were a Phoenix Monte Carlo Super Professional® and a Sunal Sound®, each having 2 × 10 fluorescent tube panels covered by transparent perspex sheets, one beneath and the other about 30 cm above the subject during exposure. The sunbed lamps, all aged for 100 h before use, were Philips UVA TL 100W/10R (R-UVA) (Fig. 1), installed in the Phoenix Monte Carlo Super Professional® bed throughout the whole 8 weeks of the study and in the Sunal Sound® for the second 4 weeks only, and Philips Trucolor 6FT 75–85 W/38 white indoor illumination lamps installed in the Sunal Sound® control bed for the first 4 weeks only. The latter lamps were chosen for their presumed insignificant UVA and UVB emissions, as assessed from their relative spectral irradiance curve (Fig. 2). Further detailed spectroradiometric assessment of the emission of each brand of lamp, perspex-filtered, was made by the U.K. National Radiological Protection Board under standard laboratory conditions, at a distance of 135 cm. Absolute irradiances in the spectral ranges 280–

TABLE 1. Burning and tanning skin types of subjects in the study

Skin type	Control	Active
I/II (burn easily)	3	18
III/IV (tan easily)	6	13

Type I—always burn, never tan;
Type II—always burn, tan on repeated sun exposure; Type III—sometimes burn, readily tan; Type IV—never burn, always tan.

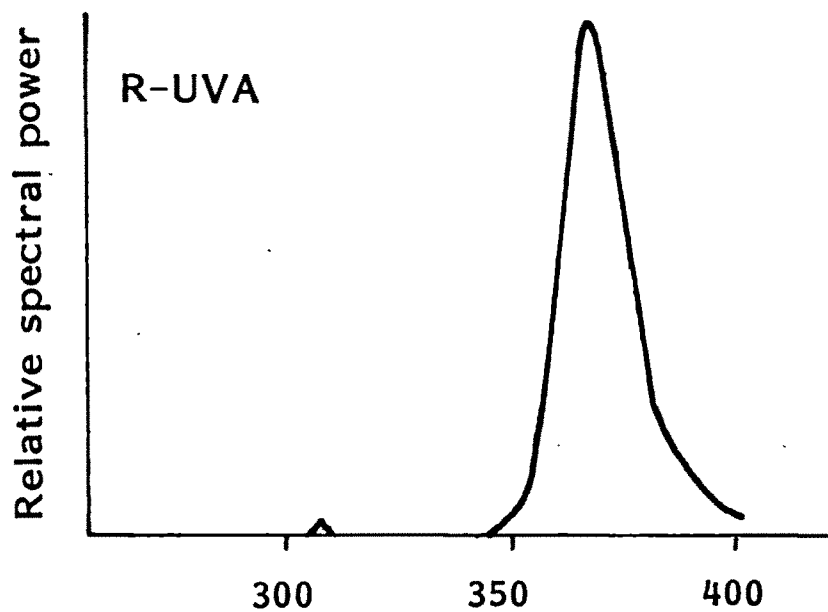


FIGURE 1. Spectral output of fluorescent Philips TL 100W/10R new UVA (R-UVA) lamps.

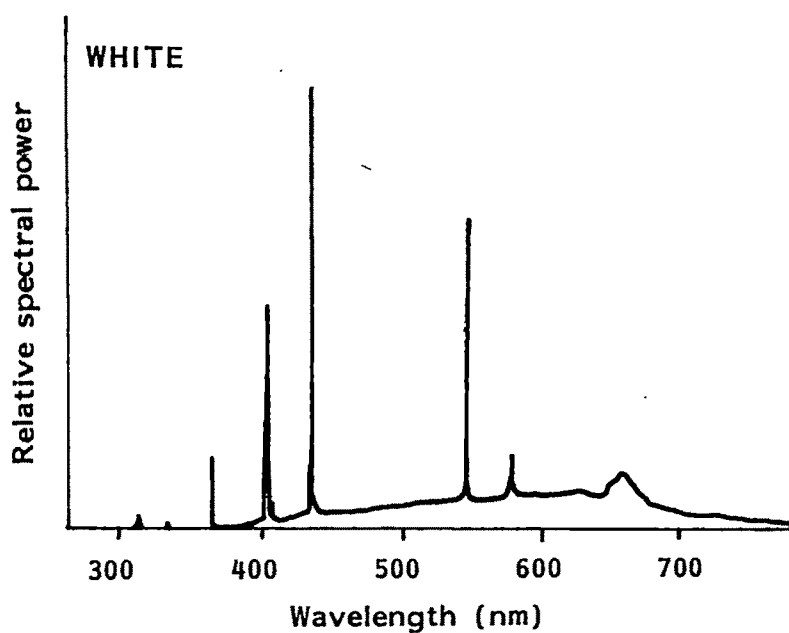


FIGURE 2. Spectral output of fluorescent Philips Trucolor 6FT 75-85W/38 white illumination lamps.

328 nm, approximating to UVB, and 328–400 nm, approximating to UVA, were determined and weighted for erythral effectiveness (Diffey BL, McKinlay AJ, personal communication). The boundary between wavebands was taken as 328 nm because several UV action spectra have been shown to change slope from oxygen independence to dependence at this point, suggesting that it may prove to be the true functional UVB/UVA boundary.^{19,20} Irradiances over the range 280–328 nm for the active lamps after attenuation by each bed's perspex covering sheet were 7.72×10^{-5} and 8.78×10^{-5} mW/cm² for the Phoenix Monte Carlo Super Professional® and Sunal Sound® beds respectively and 14.00×10^{-5} mW/cm² for the control bed, representing 7.4, 8.2 and 91.2 respectively of the total erythemogenic outputs of the beds (Table 2). Routine dosimetry of the beds to ensure constant UVA output was also undertaken weekly at the perspex lie-on surface of the beds with an International Light IL 442A meter calibrated at 366 nm. UVA irradiance remained steady in the region of 19 mW/cm² for the active bed and 0.17 mW/cm² for the control bed.

Sunbed exposures took place on Mondays, Wednesdays and Fridays for 4 weeks. The UVA exposure dose received on front and back of the body during 20 min on each occasion during the first week was about 23 J/cm² for the active bed and 0.20 J/cm² for the control, and during 30 min on each occasion during the second to fourth weeks about 34 and 0.31 J/cm² respectively, with occasional slight dosage modification where necessary. UVA protective goggles were worn during each exposure, and 25 cm² of each subject's buttock skin was shielded with aluminium foil.

Assessment of tan

Skin tanning on buttocks and on trunk and limbs 1 week after the 4-week exposure course was visually graded on a scale of 0–5 by comparison with that on the aluminium foil-shielded buttock area (Grade 0, no tan; 1, minimal tan; 2, mild tan; 3, moderate tan; 4, marked tan; 5, extreme tan).

Photoprotection

Photoprotection against erythema produced by Westinghouse FS20 fluorescent UVB and UVASUN 2000 UVA lamp exposure was assessed as the ratio of the irradiation dose required

TABLE 2. UVB and UVA spectral irradiance of active and control sunbeds and percentage of total erythemogenic radiation output from each bed emitted in range 280–328 nm

Source	Spectral Range	Perspex*	Absolute Irradiance* (mW/cm ² × 10 ⁻⁵)	Ratio (EE† weighted) 280–328 nm/280–400 nm (%)
Active bed	280–328	PM	7.72	7.4
	328–400	PM	13,200.00	
	280–328	SS	8.78	8.2
	328–400	SS	12,900.00	
Control bed	280–328	SS	14.00	91.2
	328–400	SS	173.00	

* Measured at a distance of 135 cm through perspex taken from

PM Phoenix Monte Carlo Super Professional® sunbed.

SS Sunal Sound® sunbed.

† Erythral effectiveness.

after the course to that required before to induce just perceptible confluent erythema 24 h after exposure over 3 cm² of volar forearm skin. Lamp dosimetry was undertaken with Spectroline DM and International IL 442A meters, calibrated at 300 and 366 nm respectively.

Each subject completed, under supervision, a symptom card of unwanted effects after each exposure and following the complete exposure course.

Immunological studies

Blood immune function was assessed before and 1 week after the sunbed course by measurement of antinuclear factor titre, Ro and La antibody titres, total white cell and lymphocyte counts, T-lymphocyte subsets including CD3⁺ (pan T-cell), CD4⁺ (helper T-cell) and CD8⁺ (cytotoxic/suppressor T-cell) counts and of T-cell mitogenic responses to phytohaemagglutinin (PHA), pokeweed mitogen (PWM) and concanavalin A (Con A). Blood haemoglobin, erythrocyte sedimentation rate and neutrophil count were also measured.

Epidermal Langerhans cells (LC) were counted in snap-frozen 2 mm punch biopsies of sun-protected buttock skin in six active and four control subjects from the randomly allocated group who undertook the first 4-week exposure course. One biopsy was taken before the irradiation course and two after, one of the latter from the aluminium foil-protected site. OKT6⁺ LC were immunoenzymatically labelled with immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase antibodies, and LC counted per 100 basal keratinocytes (BK).

Pityrosporum yeast counts

These were assessed before and 1 week and also 1 month after the exposure course in all subjects.²¹ Samples were obtained by gentle application of Sellotape strips to the usually heavily yeast-colonized central upper back,^{22,23} which were then placed adhesive side down on a drop of Parker's stain on a microscope slide,²⁴ left at room temperature for 24 h and examined with a $\times 40$ objective. Yeast numbers/cm² of Sellotape strip were counted over 50 high power fields (HPF) in two areas of sample and any yeast transformation to pathogenic filamentous forms was also noted. Proportions were compared by Chi-squared or Fisher's exact tests, variables measured on three or more occasions per subject by analysis of variance and mean values of continuous variables by *t*-test.²⁵ Logarithmic transformation of yeast numbers was necessary before analysis because of their skew distribution.

RESULTS

All subjects completed the exposure course. Marked tanning proved difficult to achieve. Skin in firm contact with the sunbed during exposure often remained completely pale. On other areas, compared with the aluminium foil-covered site, 16 (52%) of the 31 active but none of the nine control subjects received mild buttock tanning, seven (22%) received a moderate tan and eight (26%) a minimal tan (mean 2.12, mild, range 1-3) ($P < 0.05$) (Fig. 3a). Twenty-one (68%) of the active subjects received moderate general tanning on previously sun-exposed areas and eight (26%) mild tanning, while two (6%) received a marked general tan (mean 3.11, moderate, range 2-4) ($P < 0.05$). Control subjects also unexpectedly received a slight general tan (mean 1.67, minimal to mild, range 1-2) ($P < 0.05$; and $P < 0.05$ compared with the active group) (Fig. 3b).

The protection factors against UVB and UVA erythema found in active subjects after exposure were low compared with the pre-irradiation values of 1. Control values were lower still and not significant. The value against UVB erythema for active subjects was 3.2 ± 0.3 (mean $\pm$ SEM) (range 1-6) ($P < 0.05$) and for control 1.6 ± 0.2 (range 1-2), and against UVA

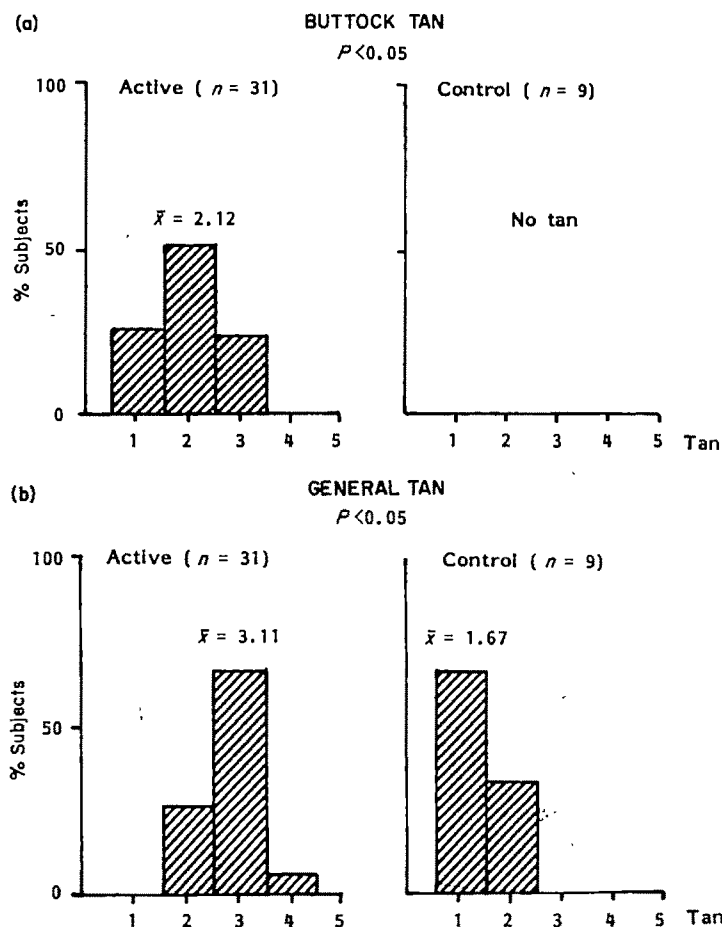


FIGURE 3. Cutaneous tanning by active and control sunbed exposure courses on exposed buttocks (3a) and on trunk and limbs (3b) compared with that on buttock skin protected from irradiation throughout sunbed exposure course (n = number of subjects, $\bar{x}$ = mean degree of tanning in active and control groups).

erythema for active subjects 1.9 ± 0.1 (range 1-4) ($P < 0.05$) and for control 1.2 ± 0.1 (range 1-2). The protection factors achieved against UVB and UVA erythema in active subjects were both significantly greater than those in control subjects ($P < 0.05$ in each case).

Early adverse reactions that were significantly more common in active subjects were pruritus, erythema, dryness of skin, freckling, burning sensation and polymorphic light eruption, while lightheadedness, nausea, fatigue and in one case claustrophobia appeared to be effects of lying on the unit rather than of irradiation (Table 3). Pruritus occurred mostly on the buttocks and legs within several hours of exposure and caused only mild to moderate discomfort, subsiding by next day, but in one subject it prevented sleep the night after exposure unless relieved by chlorpheniramine. Erythema following treatment was often generalized and usually most marked immediately, especially at contact sites over the back, but caused little discomfort and faded within hours. Skin dryness was often widespread and persisted for days to weeks, particularly on the legs. Polymorphic light eruption (PLE) defined as pruritic papules and

TABLE 3. Acute adverse effects following either single sunbed exposure or the complete exposure course for all subjects in the study

	Number of subjects affected	
	Active (n=31)	Control (n=9)
Pruritus	27*	0
Erythema	22*	0
Freckling	17†	0
Dryness of skin	15†	1
Burning sensation	15†	1
Polymorphic light eruption	13†	0
Lightheadedness	11	4
Nausea	4	1
Fatigue	4	3
Claustrophobia	0	1

* $P < 0.01$, † $P < 0.05$ for difference in incidence between groups.

plaques appearing on exposed skin within hours of exposure and lasting for hours to days occurred in 13 active subjects, five with a previous history of PLE. Four of the 13, however, were affected only on the lateral aspect of the heels. Biopsy from one PLE-like plaque supported the clinical diagnosis of PLE.

Buttock LC counts per 100 BK of exposed skin in six active subjects dropped after exposure from 12.5 ± 1.8 (mean $\pm$ SEM) to 8.4 ± 1.5 ($P < 0.05$) and in four control subjects from 12.8 ± 1.4 to 6.5 ± 0.9 ($P < 0.005$) but remained unchanged in protected skin at 12.4 ± 1.0 for the active and 12.0 ± 2.5 for the control subjects. There were no significant differences in numbers between active and control groups either before or after irradiation.

Haemoglobin, total white cell, polymorph, and lymphocyte counts, and lymphocyte proliferative responses to PHA, PWM and Con A showed no significant changes in active or control subjects. Blood $CD3^+$ (pan T-cell) and $CD4^+$ (helper T-cell) lymphocyte subpopulations were both reduced after the irradiation course in active subjects from 2.0 ± 0.1 (mean $\pm$ SEM $\times 10^6/\text{ml}$) to 1.6 ± 0.1 ($P < 0.05$) and 1.8 ± 0.1 to 1.3 ± 0.1 ($P < 0.01$) respectively, and in control subjects from 1.7 ± 0.2 to 1.3 ± 0.2 ($P < 0.05$) and 1.5 ± 0.2 to 1.0 ± 0.2 ($P < 0.001$) respectively (Table 4). $CD8^+$ (cytotoxic/suppressor T-cell) counts were also reduced in active and control subjects but not significantly as was the $CD4^+/CD8^+$ ratio from 2.9 ± 0.1 to 2.4 ± 0.1 ($P < 0.1$) in the active but not the smaller control group. No other changes in blood immunological or haematological data were noted.

Geometric mean total *Pityrosporum* yeast counts/cm² (total *P. ovale* and *P. orbiculare* forms) in the active subjects were reduced from 4281 to 2678 one week after the course ($P < 0.05$) and 2374 4 weeks after the course ($P < 0.05$), and in the control subjects from 5077 to 2832 1 week after ($P > 0.05$) and to 2641 4 weeks after the course ($P < 0.05$) with no significant differences between the active and control groups either before or after irradiation. No pathogenic filamentous forms were seen.

TABLE 4. Mean ($\pm$ SEM) of haematological and blood immunological data for active and control groups before and 7 days after sunbed exposure course

	Active (24)		Control (9)	
	Before	After	Before	After
CD3 ⁺ ($\times 10^6$ /ml)	2.0 (0.1)*	1.6 (0.1)	1.7 (0.2)*	1.3 (0.2)
CD4 ⁺ ($\times 10^6$ /ml)	1.8 (0.1)†	1.3 (0.1)	1.5 (0.2)†	1.0 (0.2)
CD8 ⁺ ($\times 10^6$ /ml)	0.7 (0.1)	0.6 (0.1)	0.6 (0.1)	0.4 (0.1)
CD4 ⁺ /CD8 ⁺ ratio	2.9 (0.1)	2.4 (0.1)	2.6 (0.2)	2.5 (0.4)

* $P < 0.05$.

† $P < 0.01$ for differences between groups.

DISCUSSION

Our double-blind controlled study of the short-term effects on normal subjects of a course of exposure to sunbeds containing UVA lamps emitting only minute amounts of UVB radiation has largely reproduced earlier results from lamps of greater UVB output, and shown no obvious advantage of the new lamps over the former. It is possible that the minimal persisting UVB output alone could still be responsible for at least some of the effects noted, particularly on T-lymphocyte subsets, LC and less importantly *Pityrosporum* yeast counts, and that lamps with even less emission below 330 nm might avoid them, but it seems unlikely that long term adverse effects would be altered by such change.^{8,9,10,12}

Only a mild tan was induced on the previously untanned buttocks of UVA exposed subjects. Most of the subjects achieved moderate tanning elsewhere, apparently by an accentuation of pre-existing tans. Tanning failed to occur at all over bony lumbosacral prominences directly in contact with the sunbed in some subjects, probably because of relative tissue hypoxia at these sites.²⁰

The exposure led to only mildly increased photoprotection against UVB erythema and even less against UVA. Similar UVA-induced photoprotection of less than a factor of 4 against UVB has been previously reported,^{26,27} although better protection has also been noted^{28,29} possibly because of higher irradiation doses. A course of sunbed exposure is unlikely to provide much protection against the effects of later UV exposure.

Adverse effects were common in the active subjects. Pruritus was particularly frequent, possibly as a result of increased histamine activity in irradiated skin,³⁰ since irradiation apparently has little direct effect on vessels and nerves likely to be associated with pruritus.³¹ Erythema occurred in the majority of active subjects but not in controls immediately after exposure and lasted for up to several hours;³²⁻³⁴ it is presumably the result of UVA induced inflammation.³⁵ Dryness of the skin occurred in about half the active subjects but not in controls, and may be a radiation effect on cutaneous lipid metabolism. PLE affected just under half the active subjects but no controls, all with negative antinuclear, Ro and La antibodies indicating that it may be induced by UVA.³⁶ Four subjects with no previous history of PLE developed the eruption on the lateral aspects of the feet only. Freckling also commonly occurred, not apparently previously reported in this mild form, although more clearly defined

sunbed lentigines have been noted.¹⁶ Other side-effects were not significantly more frequent after active exposure than control exposure and presumably resulted from warmth or merely from lying on the bed. Subjective feelings of well-being occurred approximately equally in both active and control groups, and any beneficial psychological effects in sunbed users³⁷ seem most likely to be the result of relaxation and pampering rather than of irradiation.

Epidermal LC numbers were reduced after the irradiation course in both active and control subjects. LC decrease may also occur during PUVA therapy,^{38,39} but the similar results for both active and control groups in this study suggest that the approximately equal, small but significant UVB outputs from both UVA and white fluorescent lamps were responsible, being about 10 and 90% respectively of each lamp's estimated erythemally effective emission, even after perspex covering-sheet attenuation (Table 2). A recent report shows that similar solarium-induced epidermal LC depletion may be prevented by UVB filtering during exposure.⁴⁰

We also confirmed that T-lymphocyte subset changes occur in active subjects.⁷ These included a reduction in CD4⁺ (helper T-cell) numbers, which was significant in this study. However, instead of increases in CD3⁺ (pan T-cell) and CD8⁺ (cytotoxic/suppressor T-cell) numbers, we found a statistically significant decrease in the former and a trend towards decrease in the latter. On the other hand there was a marked trend ($P < 0.1$) towards a significant decrease in the CD4⁺/CD8⁺ ratio at the end of the active irradiation course. We also found similar changes in the control subjects except in the CD4⁺/CD8⁺ ratio, strongly suggesting that the reductions were again UVB effects, as postulated above for LC. It may, therefore, be that the higher UVB output or more frequent exposures in the previous study were responsible for the slightly different results,⁴¹ but it is apparent that sunbed exposure even with very low UVB emission can lead to significant T-cell effects.

Total UVB exposure from active and control lamps throughout the course amounted to about one minimal erythema dose, a quite small absolute exposure but perhaps more active than expected before the study because fractionated.⁴² It is possible that regular exposure to office lighting resembling our control lamps, although far from similar to sunbed irradiation, could also cause LC and T-lymphocyte disturbances with potential impairment of immune surveillance. Hence, recent epidemiologic reports of possible increased malignant melanoma risk in office workers^{43,44} may warrant further consideration.

Observations^{14,15} that sunbed users may have an increased susceptibility to pityriasis versicolor infection were not supported by our results, since *Pityrosporum* yeast numbers were in fact reduced equally in both groups, probably by UVB. Thus, impaired tanning of already affected sites is more likely to explain the apparent onset of new infections following sunbed exposure.

The allocation of active and control subjects was random but since subjects were allotted to the groups in sequential fashion there was a small mismatch of skin types, with proportionately more fair-skinned subjects in the active group (Table 1). This mismatch may have slightly diminished the degrees of tanning and photoprotection achieved in the active group, but the results were such that any slight improvements in the active group would be unlikely to alter significantly the overall findings. More adverse effects could have been expected in the active group because of its preponderance of skin types I/II over III/IV, and separate statistical comparison of the two treatment groups was therefore undertaken for each skin type. This only showed significant increases in the active group for pruritus and erythema ($P < 0.05$ for both skin types in each case), while freckling, burning sensation, dryness of skin and polymorphic light eruption no longer reached significance. However, the extreme preponderance of the latter effects in the active group (Table 3) strongly suggests that the active bed alone was responsible for them.

This study has demonstrated that a month-long, three times weekly course of cutaneous sunbed exposure to UVA lamps emitting very small amounts of UVB radiation leads to only moderate tanning and protection against later UV-exposure, whereas annoying minor acute adverse effects are common and potentially serious disturbances in cutaneous LC and blood nbT-cell subsets occur. In spite of this, however, commercial and private use of UVA sunbeds is increasing and the insignificant advantages and possible risks of such equipment must be further emphasized. Our data strongly reinforce the statement of the Photobiology Task Force of the American Academy of Dermatology² that use of high-intensity UVA light sources for cosmetic tanning is harmful and should be discouraged.

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Actinic reticuloid: immunohistochemical analysis of the cutaneous infiltrate in 13 patients

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SUMMARY

An immunohistological study on cryostat sections of skin biopsies in 13 patients with actinic reticuloid has been performed using a panel of 21 monoclonal antibodies against lymphoid and non-lymphoid infiltrate cells. The infiltrate consisted of activated T cells, numerous histiocytes and macrophages, and small numbers of B cells. In 10 out of 13 patients the majority of the lymphocytes expressed the phenotype of suppressor cells. The number of Leu 8⁺ cells was inversely proportional to HLA-DR expression by the dermal infiltrate, which suggested a negative correlation between a state of activation and the concentration of Leu 8⁺ cells. There was a striking number of IgE bearing dendritic cells in the dermis associated with elevated serum IgE levels.

Actinic reticuloid (AR) is a rare severe chronic photodermatosis occurring in elderly male patients. Lesions are not only confined to light-exposed skin but can also extend to covered skin areas and episodes of erythroderma are not uncommon.¹

The dermal infiltrate in AR has been well documented by light- and electron microscopy^{1–3} but immunohistological studies are few. In a study which employed a large panel of monoclonal antibodies against lymphoid cells and their subsets in five AR patients, an excess of T-helper/inducer cells was found in two patients while in the other three patients the numbers of helper and suppressor cells were approximately equal.⁴ However, in a recent study of 16 Dutch patients somewhat different results were obtained.⁵ Cells with a suppressor-cell phenotype were the preferential cell type in the dermal infiltrate in most patients (10 of 13 cases studied). In the present study we have extended these findings by using a panel of 21 monoclonal antibodies against lymphoid cells and non-lymphoid cells.

TABLE 1. Clinical data of 13 patients with actinic reticuloid

Patient	Age at diagnosis (years)	Sex	Spectrum of photosensitivity	Extensions of lesions	Preferential phenotype skin lymphocytes
			A,B,C ₂ = ultraviolet A,B,C ₂		
1	68	M	B ₂ (A+VL)* (315-780 nm)	E	TS
2	63	M	C+B+A+VL (380-450 nm)	E	TS
3	49	M	B+A+VL (380-600 nm)	E	TS
4	69	M	C+B+A+VL (380-780 nm)	E	TS
5	76	M	C+B+A+VL (380-600 nm)	L+NL	TS=TH
6	65	M	B+A+VL (380-450 nm)	L+NL	TH
7	53	M	B+A+VL (380-600 nm)	E	TS
8	52	M	B+A+VL (380-450 nm)	E	TH
9	63	M	B ₂ (A+VL)†	E	TS
10	69	M	C+B+A+VL (380-600 nm)	L+NL	TS
11	62	M	LC+B+A+VL (380-450 nm)	E	TS
12	58	M	C+B+A+VL (380-450 nm)	L+NL	TS
13	75	M	B+A+VL (380-600 nm)	E	TS

* Combination of UVA plus VL tested, not the wavelengths separately.

† Not possible to test all wavelengths of visible spectrum.

VL = visible light with range of photosensitivity between parentheses

E = erythroderma.

L+NL = lesions on light-exposed skin with extension to non-exposed areas.

TS = T-suppressor cells.

TH = T-helper cells.

METHODS

Patients

AR was diagnosed in 16 patients admitted to the Department of Dermatology between 1975 and 1987. All the patients had chronic infiltrated papules and plaques or a diffusely infiltrated skin particularly in light exposed areas and often extending on to the covered areas. Prolonged episodes of generalized erythroderma occurred in 10 of the patients. A severe photosensitivity was present in all cases, involving the UVB and UVA ranges as well as part of the visible spectrum. In fully developed lesions the dense dermal infiltrate consisted mostly of lymphocytes with convoluted nuclei resembling cutaneous T-cell lymphoma. A detailed study of these patients will be published elsewhere.⁵ The main clinical data are summarized in Table 1. In 13 patients, biopsies of clinically active lesions were available for the present study.

Immunohistology

Punch biopsies of infiltrated lesional skin were snap frozen in liquid nitrogen and stored at -90°C until processing. A sensitive three-stage immunoperoxidase technique was used.⁶ A list of the monoclonal antibodies used in the present study is shown in Table 2.

A helper or suppressor cell predominance was determined in different areas of each biopsy, by estimating the number of CD8⁺ cells per section, compared with the total number of CD3⁺ cells.

TABLE 2. Monoclonal antibodies used in this study

Antibody	CD code	Specificity in cutaneous infiltrates	Source	Titre used
OKT 6	CD 1	Langerhans cells,	Ortho	1:100
Leu 1	CD 5	T cells	BD	1:50
Leu 2a	CD 8	Suppressor/cytotoxic T cells	BD	1:100
Leu 3a	CD 4	Helper/inducer T-cells	BD	1:100
Leu 4	CD 3	T cells	BD	1:100
T11	CD 2	E-rosette receptor	CLB	1:20
Leu 14	CD 22	B cells	BD	1:50
Pan B	CD 22	B cells	DAKO	1:5
Ki-1	CD 30	Hodgkin and Reed-Sternberg cells	DAKO	1:5
PC	CD 26	Cell proliferation associated nuclear antigen	DAKO	1:5
Leu M1	CD 15	Monocytes, granulocytes, Reed-Sternberg cells	BD	1:25
M 718	—	Macrophages	DAKO	1:40
HLA-DR	—	B cells, activated T cells, monocytes, Langerhans cells	BD	1:500
IgE	—	IgE molecule	CLB	1:100
TFR	—	Transferrine receptor	DAKO	1:5
DRC-1	—	Dendritic reticulum cells	DAKO	1:20
Leu 7	—	Natural killer cells	BD	1:5
Leu 8	—	Subset T cells	BD	1:5
Leu 11b	CD 16	Natural killer cells, subset T cells	BD	1:5
CR 1	CD 35	C ₃ b and C ₄ b receptor, B cells, myelomonocytic cells	BD	1:5
IL-2R	CD 25	Interleukin 2 receptor	DAKO	1:5

Ortho = Ortho Diagnostics.

BD = Becton Dickinson.

CLB = Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

DAKO = Dakopatts.



FIGURE 1. Epidermal and dermal CD₁ positive cells in lesional skin of actinic reticuloid (original $\times 100$).

RESULTS

Keratinocytes

In most patients a large proportion of the basal keratinocytes showed an intense staining with the monoclonal antibody against the cell proliferation associated nuclear antigen (CD26). In two patients only a few of the basal cells were positive. The epidermis showed intercellular staining with anti-HLA-DR in 11 patients either in isolated foci or in large areas. These specimens also showed epidermotropism of lymphocytes, whereas in two other patients neither epidermotropism nor intercellular HLA-DR were present.

Langerhans cells

In all biopsies the number of epidermal CD1⁺ dendritic cells (Langerhans cells) was reduced. The remaining cells were altered in morphology with plump and shortened dendrites that were fewer than normal. CD1⁺ dendritic cells were also present in the dermal infiltrate either as single cells, or, more often, in small or large clusters. The latter were particularly present in the upper dermis (Fig. 1).

Lymphocytes

In 10 out of 13 patients, more than 50% of the dermal lymphocytes expressed the phenotype of T-suppressor/cytotoxic cells, mostly CD8, which comprised about 90% of the lymphoid cells in

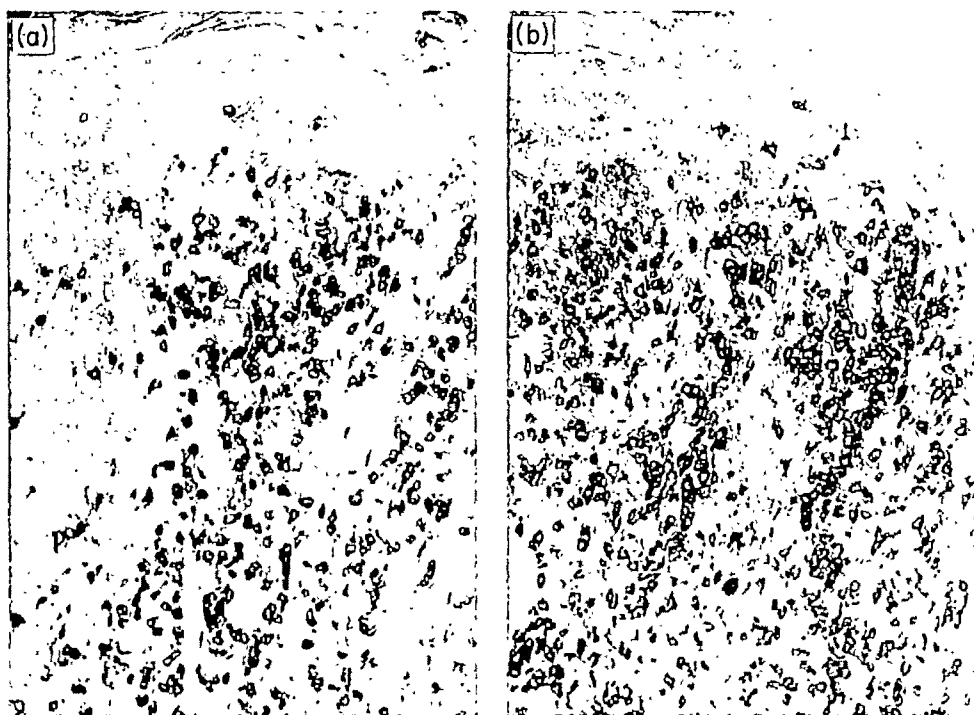


FIGURE 2. (a) Dermal infiltrate of actinic reticuloid, stained with the pan-T-cell antibody CD₃ (original $\times 100$); (b) the same section, stained with CD₈. The majority of the lymphocytes expresses the phenotype of cytotoxic/suppressor T cells.

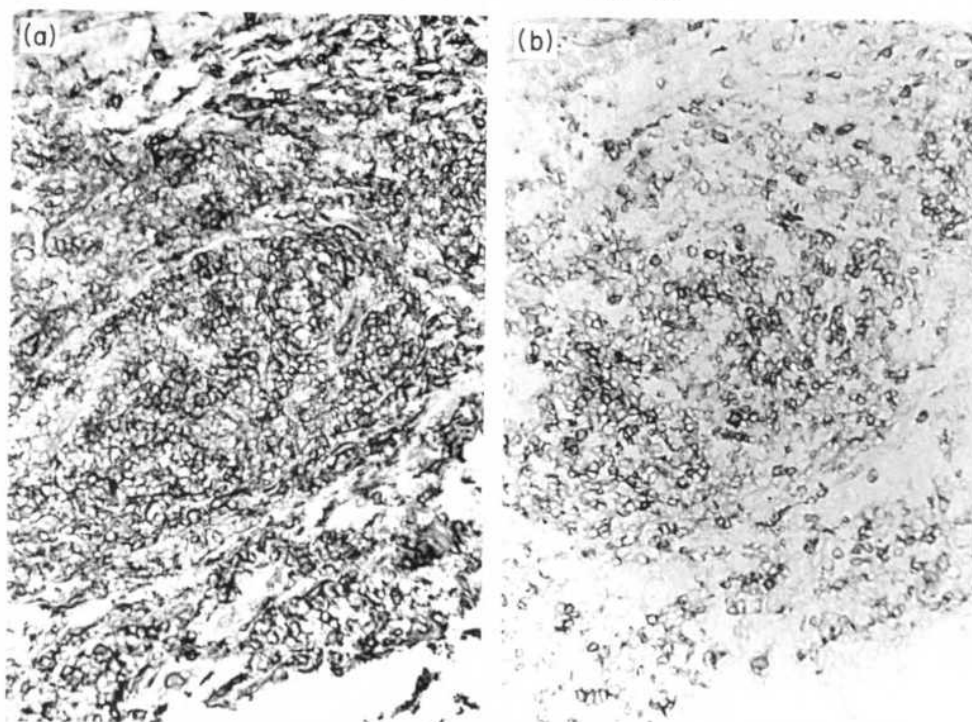


FIGURE 3. (a) Detail of the dermal infiltrate of actinic reticuloid, stained with anti-HLA-DR. Foci of unstained lymphocytes are present among the infiltrate (original $\times 250$); (b) the same section, now stained with the antibody Leu 8. Many positively staining cells are present in this area.

the most photosensitive erythrodermic patients (Fig. 2a and b). In two patients a helper/inducer cell predominance was found and in one patient numbers were about equal.

The majority of the lymphoid cells was also HLA-DR⁺. Up to 10% of the lymphocytes were positive for the cell proliferation associated antigen CD26. Less than 10% expressed the interleukin-2 receptor and the transferrine receptor. In all cases but one, B-cells occurred only sporadically. In one patient focal accumulation of positive cells was seen with small numbers scattered throughout the infiltrate.

About 10–25% of the total number of T cells were positive for the antibody Leu 8. Higher numbers were observed in two patients (30–40%). It was striking that the number of Leu 8⁺ cells was inversely related to the expression of HLA-DR; the lowest numbers were observed in those patients who displayed the highest HLA-DR expression (Fig. 3a and b).

Histiocytes/macrophages

Large numbers of M718⁺ dendritic cells contributed to the infiltrate and were also present in the surrounding collagen in all patients. Specimens from nine patients revealed numerous IgE⁺ dendritic cells throughout the papillary and reticular dermis (Fig. 4). They clearly outnumbered the number of mast cells. There was a rough correlation with the titre of serum IgE in the majority of patients in whom this was determined. Patients with high serum IgE levels tended also to have the highest numbers of IgE⁺ cells in the dermis. No double staining technique was

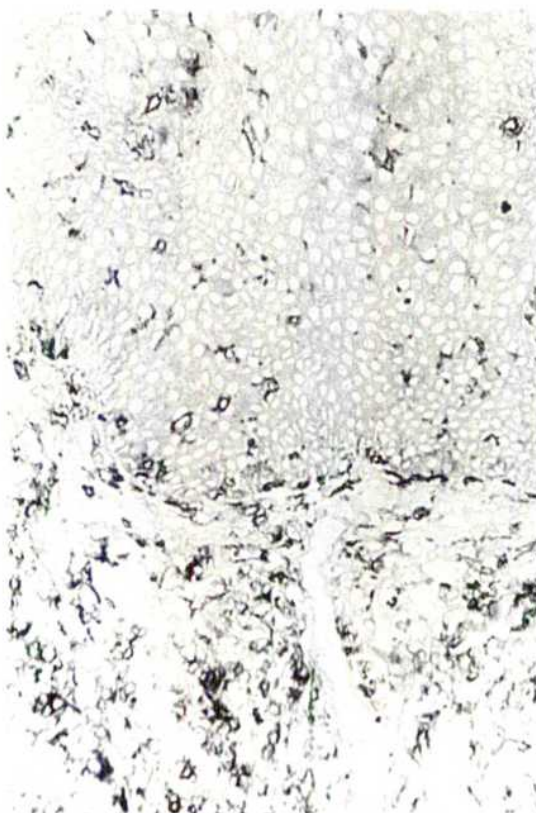


FIGURE 4. Lesional skin of actinic reticuloid stained with anti-IgE. Many IgE⁺ dendritic cells are scattered through the dermis. Note the presence of positively stained short dendritic processes in the epidermis (original $\times 160$).

done to determine whether Langerhans cells were IgE⁺, although in a few cases positively staining dendrites in the epidermis were observed, suggesting this might be the case.

Miscellaneous

CD15⁺ dendritic cells were occasionally seen in all patients, scattered through the upper dermis. Natural killer cells (Leu 7⁺, CD16⁺) were absent as were CD35⁺ cells and dendritic reticulum cells. CD30⁺ cells were sporadically present in three patients. Their morphology was comparable with small immunoblasts.

DISCUSSION

The present study shows a fairly uniform picture of the dermal infiltrate in actinic reticuloid. In 10 out of 13 patients the majority of the dermal lymphocytes expressed the phenotype of suppressor T cells. In most reactive inflammatory reactions a helper T cell predominance is found.⁷ A reversed T-helper/suppressor cell ratio is rather uncommon in these conditions, but has been reported in chronic graft-versus-host disease,⁸ cases of lichen planus,⁹ Mucha-

Habermann disease¹⁰ and also in a small percentage of cutaneous T-cell lymphomas, especially those with an aggressive clinical course.¹¹ A suppressor cell predominance in the skin in erythrodermic patients, in whom phototesting cannot be done, strongly suggests a diagnosis of AR.

It is known that ultraviolet radiation generates suppressor T lymphocytes¹² and the highest numbers were observed in the most photosensitive patients, but it is difficult to imagine how this phenomenon can play a role in AR because these patients avoid exposure to sunlight.

In polymorphic light eruption a predominance of suppressor cells has been reported in lesional skin in some cases,¹³ but other investigators could not confirm these findings in artificially induced skin lesions.¹⁴ Ultraviolet-B exposures have been given as a tolerance induction therapy in the patients reported here, but all the biopsies were taken before starting this treatment.

Induction of suppressor T cells can also be observed when a contact allergen is given to Langerhans cell deficient skin.¹⁵ Contact dermatitis is frequently found in AR¹⁶ and was also observed in the patients of the current study.⁵ Although the number of epidermal Langerhans cells was diminished, complete absence of these cells was never seen.

The histological features of AR do not differ significantly from chronic contact dermatitis. Immunohistological studies of allergic contact dermatitis and irritant contact dermatitis were only performed on acute and short lasting reactions.¹⁷ These revealed variable patterns with helper cell predominance in some, and suppressor cell predominance in others.

In almost all the patients very low numbers of Leu 8⁺ cells were present. Only in two patients were higher numbers observed (30–40%). Low expression of Leu-8 is more common in mycosis fungoides than in reactive inflammatory processes.^{18,19} A high proportion of Leu 8⁺ cells was thought to be indicative of a relatively benign disorder.²⁰ However, low numbers of Leu 8⁺ cells have also been observed in lesions of discoid lupus erythematosus.²⁰ Our study demonstrated that the number of Leu 8⁺ cells was proportional to the amount of HLA-DR expression of the dermal infiltrate. It suggests that there might be a link between the state of activation of the lymphoid cells and the presence of Leu 8⁺ cells.

Low numbers of CD26⁺ lymphocytes were seen in all patients. Markers of cellular activation, such as the interleukin-2 receptor and also HLA-DR, were present on the majority of the lymphocytes and also on many dendritic cells in the dermis.

The high concentration of IgE⁺ dendritic cells in the dermis is also found in skin lesions of atopic dermatitis patients (unpublished observations). Both categories have elevated serum levels of IgE. This has also been reported in actinic reticuloid,²¹ polymorphic light eruption²² and some lymphoproliferative disorders such as Hodgkins disease and mycosis fungoides.^{23–25} The high serum IgE may result from diminished T lymphocyte suppression,²¹ though this does not apply in the patients reported here because the majority had a suppressor cell predominance in the skin and also sometimes in the peripheral blood.⁵ Another explanation has to be found to elucidate this phenomenon in AR.

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Cutaneous reactive hyperaemia: racial differences induced by corticoid application

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SUMMARY

The postocclusive hyperaemic reaction before and after a single 1 h application of a potent corticoid (clobetasol 0.05% in ointment) was recorded by means of laser Doppler velocimetry in order to elucidate different racial responses in skin vasoconstriction. Fourteen young male subjects entered the study (eight Caucasians, six Blacks). Reactive hyperaemia was recorded after 4 min of occlusion of the forearm blood flow. The following parameters of the hyperaemic reaction were investigated: area under the curve response, peak response, rise of blood flow slope to peak and decay of blood flow slope after peak. Different responses were recorded in the Black subjects after the vasoconstrictive stimulus compared to the Caucasians, namely: decreased area under the curve response ($P < 0.04$); decreased peak response ($P < 0.01$); decreased decay slope after peak blood flow ($P < 0.04$). These data are consistent with a different reactivity of blood vessels in black skin and possibly not related to the transcutaneous penetration of the chemical compound.

The vasoconstriction assay is widely used to assess the transcutaneous penetration of corticoids. This technique, developed by McKenzie and Stoughton,¹ is based on the ability of glucocorticoids to elicit a blanching reaction after topical application. The degree of vasoconstriction usually parallels the anti-inflammatory effect. The blanching reaction is usually scored visually, rendering this technique sensitive to subjective evaluation. The non-invasive, objective quantification of skin blood flow was demonstrated in a preliminary report, to be ineffective in monitoring and quantifying corticoid induced vasoconstriction.² To overcome these problems and allow an objective evaluation of blanching, Bisgaard *et al*³ recorded, by means of laser Doppler velocimetry, the reactive hyperaemia induced by arterial occlusion.

Reactive hyperaemia is the temporary increase of blood flow in a tissue when the circulation is released after a period of vascular occlusion. Two theories have been advocated to explain this phenomenon: the myogenic and the metabolic theory. The former, introduced early this century,⁴ reflects the concept that arteries and smooth muscles react to stretching by contraction

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and to decreased tension by relaxation; the latter is explained on the basis of the release of a vasoactive mediator.⁵ The accumulation and the removal of this vasoactive metabolite is proportional to blood flow.⁶ Neither theory has been completely validated in relation to the skin response to blood occlusion.

We have demonstrated racial differences between Blacks and Caucasians in the development of an erythematous reaction after chemical injury.⁷ Blacks developed less erythema than Caucasians (detected as an increase of blood flow) after exposure to the same amount of sodium lauryl sulphate. In this study, we investigated the characteristics of the cutaneous reactive hyperaemia in the two races before and after a vasoconstrictive stimulus induced by the topical application of clobetasol propionate 0.05% ointment.

METHODS

Fourteen healthy males (mean age 27 ± 3) (six Blacks, eight Caucasians) were studied, having given informed consent. The Blacks had dark black skin and had African ancestors, whilst the Caucasians were of European ancestry. Measurements were performed continuously on the forearm where blood flow was monitored with a laser Doppler velocimeter (MedPacific, LD 5000, Seattle, Washington U.S.A.) before, during and after occlusion (Fig. 1). A helium neon fibre of 632.5 nm wavelength is applied to the skin via a small probe. The incident light strikes moving blood cells (erythrocytes) and is reflected with a shift in frequency. The amount of this shift, proportional to the blood flow is displayed in mV and recorded with a chart recorder. The laser Doppler probe was attached to the forearm surface by double sided adhesive tape. Occlusion was obtained by applying a pneumatic cuff to the forearm inflated to a pressure of 280 mm Hg for 4 min. One hour after the baseline recording a plastic occlusive chamber (Hill Top,[®] Cincinnati, Ohio U.S.A.) containing clobetasol propionate 0.05% ointment was applied for 1 h on the same skin site. It is possible to detect early vasoactive effects of glucocorticoids. The dose of corticoid applied was 7.8 mg/cm^2 . Immediately after the removal of the occlusive device another measurement was repeated. Skin temperature ranged from 31 to 32.5°C and was not affected by the application of the laser light on the skin surface.

The following parameters of the cutaneous reactive hyperaemic curve were analyzed: (i) The area under the curve response calculated from the starting point of the hyperaemic response until the return of the blood flow to basal values. (ii) The magnitude of the maximum peak

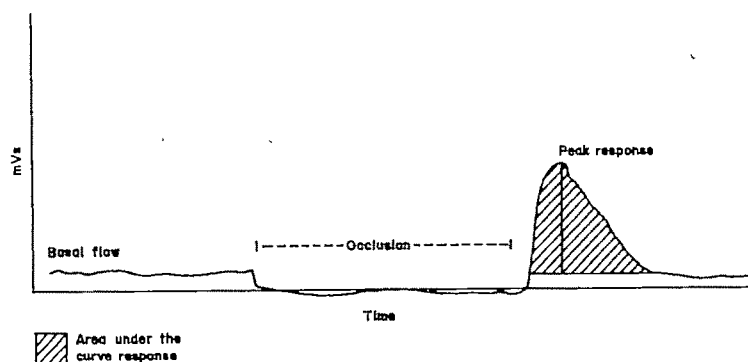


FIGURE 1. Schematic representation of laser Doppler velocimetric recording of postocclusive reactive hyperaemia in the volar forearm.

response. These values were calculated in arbitrary units following the method described by Bisgaard.³ (iii) The slope of the rise from immediately post occlusion to peak reactive hyperaemic flow, calculated by means of least squares regression of the natural logarithm of peak flow erythrocyte flux minus the erythrocyte flux at the time $t(n)$ (time of the n th sample) vs. time of the peak erythrocyte flux minus $t(n)$ (according to Wilkin⁸). (iv) The slope of the recovery from peak reactive hyperaemic flow to resting levels, calculated by least squares regression of natural logarithm of blood flow vs. time in seconds. Statistical analysis of the data was obtained using unpaired and paired Student's t -test (two-tailed). All the subjects were normotensive, and rested in the laboratory 1 h prior to the various procedures.

RESULTS

Area under the curve response

The values for the area under the curve response are described in Table 1. No significant differences were recorded between the two groups. The area under the curve decreased in both after the vasoconstrictive stimulus, but significantly only in the Black group which showed an approximately 40% reduction ($P < 0.04$).

Peak response

The peak response of the hyperaemic reaction did not reveal significant differences between the two groups. The values were lower after the treatment with the corticoid in both, but statistically significant only in Blacks ($P < 0.01$) (Table 1).

Slope of blood flow rise to peak response

The values of slope for each subject are shown in Table 2. No significant differences were recorded between the two races and after the treatment.

TABLE 1. Area under the curve and peak response of the hyperaemic reaction before and after a single occlusive application of clobetasol propionate 0.05% in Caucasians and Blacks. Values are in arbitrary chart units. Significant differences are detected in the Black group after the treatment for both the parameters investigated ($P < 0.04$ for the area under the curve, $P < 0.01$ for the peak response)

Cutaneous hyperaemic reactive response		
Caucasians basal	Area under the curve	358 ± 222
	Peak response	20 ± 10
Caucasians after treatment	Area under the curve	290 ± 189
	Peak response	19.2 ± 8.1
Blacks basal	Area under the curve	355 ± 158
	Peak response	28 ± 8.8
Blacks after treatment	Area under the curve	206 ± 115
	Peak response	14.3 ± 6.5

TABLE 2. Values of the slope of blood flow rise to peak response calculated for each subject before and after treatment. No significant differences are appreciable between the two races and following treatment

Blacks		
	Basal	After treatment
1	0.250	0.4158
2	0.2875	0.3240
3	0.1595	0.0977
4	0.1883	0.1911
5	0.2204	0.5129
6	0.2616	0.2059
Caucasians		
	Basal	After treatment
1	0.0653	0.1064
2	0.1004	0.0903
3	0.1542	0.3301
4	0.1634	0.3972
5	0.1934	0.3899
6	0.3604	0.3860
7	0.2281	0.3823
8	0.2893	0.1480

Slope of blood flow decay after peak response

The values of the slope for each subject are represented in Table 3. The decay of blood flow after peak response is similar in the two races in the baseline experiment (Fig. 2). After treatment the decay slope differs significantly only in the Black group ($P < 0.04$) (Fig. 3, Table 4).

DISCUSSION

The mechanism of reactive hyperaemia is still unclear. The metabolic theory involves the accumulation of prostaglandins formed or released in response to hypoxia or depletion of a critical substrate due to reduced tissue perfusion. This explains the greater reactive hyperaemic reaction after prolonged occlusion. Kilbom and Wennalm⁹ demonstrated that after occlusion of the forearm for 5 min, there was a relationship between the degree of hyperaemia and the increase of release of prostaglandins, which could be reduced by indomethacin pre-treatment. The reduction of the hyperaemic response induced by corticoids³ is due to the inhibition of prostaglandins secretion secondary to the anti-inflammatory action of glucocorticoids. Monitoring reactive hyperaemia after the topical application of corticoids has been proposed as a model for evaluating corticoid efficacy. A significant dose-response relationship ($P < 0.01$) was demonstrated applying four different corticoids, namely clobetasol, budesonide, hydrocortisone butyrate and hydrocortisone.³ The vascular response was inversely related to the anti-

TABLE 3. Slopes of blood flow decay calculated for each subject (Blacks, Caucasians, before and after treatment): There is a significant difference between pre- and after treatment in Blacks ($P < 0.04$) (Fig. 3)

Blacks		
	Basal	After treatment
1	-0.1668067	-0.0635611
2	-0.0451117	-0.0238832
3	-0.0591873	-0.0187219
4	-0.0603814	-0.0452353
5	-0.0520553	-0.0204626
6	-0.0589034	-0.0483183
Caucasians		
	Basal	After treatment
1	-0.0371876	-0.0261867
2	-0.079849	-0.1200777
3	-0.0403374	-0.0679623
4	-0.079849	-0.0784889
5	-0.0376784	-0.025688
6	-0.0435329	-0.027227
7	-0.0418373	-0.028408
8	-0.0285423	-0.0166029

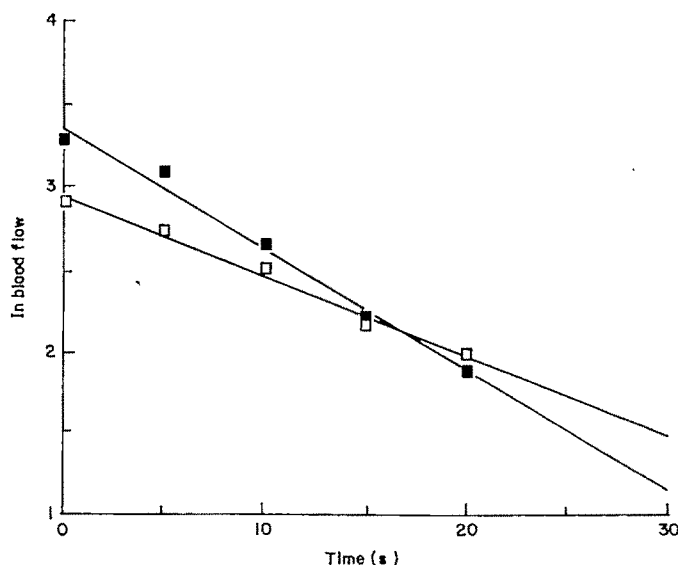


FIGURE 2. Slope of the decay of blood flow after peak hyperaemic response in Caucasians and Blacks (basal values). The trend in the two races is not significantly different. ■, average Black, $y = 3.3672 - 0.0737x$, $R = 0.99$; □, average Caucasian, $y = 2.95 - 0.0486x$, $R = 0.99$.

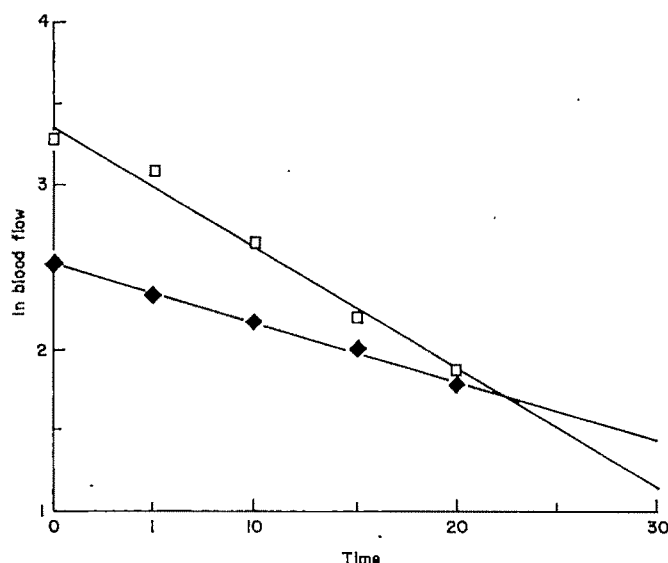


FIGURE 3. Slope of the decay of blood flow after hyperaemic peak response in Blacks. Comparison of basal vs. treated values. The slope is obtained averaging values of each subject (Table 4). Comparing the values of the slopes obtained in each subject (Table 3) a statistical significant difference is obtained in the Black group before and after the treatment ($P < 0.04$). □, average before treatment Black, $y = 3.3672 - 0.0737x$, $R = 0.99$; ◆, average after treatment Black, $y = 2.5347 - 0.0367x$, $R = 1.00$.

TABLE 4. Mean values in natural logarithm of blood flow decay after peak hyperaemic response before and after treatment

Mean values of blood flow decay after peak hyperaemic response
(natural log)

Basal					
Average Black	SD	Average Caucasian	SD	Time	
3.2879355	0.32925326	2.91920477	0.50172368	0	
3.09402192	0.28883374	2.7430363	0.48552774	5	
2.67057954	0.39397954	2.50368625	0.48990202	10	
2.20986074	0.79709134	2.15638867	0.58830209	15	
1.88649205	0.96798695	1.99748516	0.59819117	20	
After 1 h occlusion with clobetasol					
Average Black	SD	Average Caucasian	SD	Time	
2.53727933	0.6118697	2.8832257	0.41122979	0	
2.33627246	0.66902944	2.68914709	0.51226784	5	
2.16782542	0.752372	2.51456314	0.54090236	10	
2.01811338	0.70576193	2.13203218	0.90858298	15	
1.77893263	0.91991464	1.94102835	0.88071763	20	

inflammatory action of the molecule tested. The reactive hyperaemia was significantly reduced after 2 h exposure to budesonide ($P < 0.05$) and extending the time to 24 or 48 h caused a significant dose-response relation ($P < 0.05$). The involvement of cyclooxygenase products in the reactive hyperaemia was assessed in two subjects, comparing the basal response and the reaction after the injection of 50 mg indomethacin in the ipsilateral cubital vein. This produced a 40% reduction of the response in 10 experiments ($P < 0.05$). This procedure did not affect blood pressure. On the other hand, the time of arterial occlusion is critical in determining a satisfactory hyperemic reaction and an occlusion time of at least 2 min is required.

The relationship between the time of arterial occlusion and the hyperaemic reaction has been studied in detail.⁹ The forearm circulation was occluded for 0.5, 1, 2, 4, 6 min and the blood flow monitored with laser Doppler velocimetry and the slopes of rise to peak and decay of blood flow evaluated. Both slopes showed a decrease in mean values with increasing duration of occlusion, although the peak response increased proportionally with time of occlusion presumably representing the opening of less compliant microvessels that lower the overall rate of rise. The time-dependent rate of blood flow increase to peak response suggests that viscoelasticity of blood vessels determines the response. Indeed, the increased rate of rise at shorter durations of occlusion is unlikely to be due only to a vasodilator metabolite, the concentration of which should increase with longer duration of occlusion. This is consistent with the modifications induced by pressure changes in the arteriolar wall and viscoelasticity produces the reactive hyperaemic response.¹⁰

In the basal experiment, our study reveals basically the same behaviour between black and white skin. No significant differences were recorded for all the parameters investigated (Table 1, 2, Fig. 2). Different responses were elicited by the application of clobetasol 0.05% for 1 h. In the Caucasian group, the area under the curve was reduced, although not significantly. The peak response was unchanged (Table 1). In Blacks, these parameters were greatly reduced. Values were significantly lower ($P < 0.04$, area under the curve; $P < 0.01$, peak response) (Table 1) after the application of the corticoid.

The slope of blood flow rise did not reveal differences between races and before and after the treatment (Table 2). The different reactivity of skin vessels in the races was confirmed by the behaviour of the decay of blood flow after the peak response; although in the baseline experiment the slopes were not significantly different (Tables 3, 4, Fig. 2), the treatment induced a different response in the Black group ($P < 0.04$) with decreased levels of response (Fig. 3).

These data suggest a decreased reactivity of blood vessels in Blacks during the hyperaemic reaction, mainly related to the recovery of blood flow after the peak response, following the application of a vasoconstrictive stimulus.

It is possible that there is an increased transcutaneous penetration in Blacks of the corticoids but previous studies show that it is not increased when compared with Caucasians. *In vivo* penetration of diflorasone acetate is the same in Caucasians and Blacks;¹¹ *in vitro*, permeation of fluocinolone acetonide was higher through normal white skin through black skin.¹² Furthermore, the penetration of other compounds is decreased in Blacks.^{13,14}

Our data could be explained on the basis of a different 'reactivity' of black blood vessels due to an increased removal of the vasodilator metabolite which accumulates during the occlusion time or to a different viscoelastic response of the arteriolar wall in Black race. This is consistent with our previous finding⁷ reporting a decreased susceptibility of Blacks in developing erythema after exposure to low concentrations of irritants. In fact, 2% sodium lauryl sulphate (SLS) elicited the same erythematous reaction in Caucasians and Blacks (as long as measured with laser

Doppler velocimetry), but 0.5% SLS produced significant reaction only in Caucasians. Earlier works, performed with visual methods of assessment of blood flow, reported decreased minimal perceptible erythema in Blacks.¹⁵ These differences reported were related to anatomical and structural differences in Black stratum corneum,¹⁶ but in the light of the present work we can assume that other functional factors are involved in determining the different vascular response in Blacks.

The stiffness and the compliance of the ground substance and of the tissues surrounding the vessels should be taken into account. Occlusion of the arterial supply has been demonstrated to increase the drainage of extravascular fluid across the vessel.¹⁷ Ground substance could be influenced by adrenal corticoid activity, but as far as we know, no data are reported on racial differences in ground substance composition. Attempting to understand cutaneous function via models of physical anthropology, although in an early stage, may offer a fruitful future. These striking differences in blood vessel reactivity must now be further investigated to define the pathophysiology and clinical implications.

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Skin disease in haemophiliacs with and without antibodies to the human immunodeficiency virus (HIV): further evidence of altered disease behaviour in different risk groups?

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SUMMARY

Forty-one patients routinely attending the Oxford Haemophilia Centre entered a controlled, blind investigation in order to determine whether HIV antibody status was related to the presence of skin disease. Twenty-four of the 41 patients (58.5%) were HIV antibody positive and none had any general symptoms. Comparison of the HIV antibody positive group with the HIV antibody negative group and with non-haemophiliac controls showed an increased prevalence of four HIV-associated dermatoses: 11 patients had seborrhoeic dermatitis (10 HIV antibody positive and one HIV antibody negative ($P < 0.05$ χ^2 test)), eight patients had folliculitis (six HIV antibody positive), four patients had mucocutaneous candidiasis, all were HIV antibody positive, and three patients had onychomycosis, all were HIV antibody positive. None of these conditions was seen in a group of 16 non-haemophiliac controls. These findings are different from those reported from a similar study of comparable groups of homosexual men and these results may be further evidence to support the belief that the behaviour of HIV infection differs between haemophiliacs and other risk groups.

Haemophiliacs are dependent upon transfusions of blood products in order to remain well. Commercial clotting factor concentrates are prepared from many thousands of blood donations and haemophiliacs have always been at risk of infection with blood-borne viruses. Viral hepatitis may be responsible for considerable morbidity and mortality,¹ but the Human Immunodeficiency Virus (HIV) poses a more serious threat.

Nearly 60% of severe British haemophiliacs are seropositive for HIV antibodies.² In the United States between 75% and 90% of severe adult haemophiliacs, and 58% of children with severe haemophilia are HIV antibody positive.³ Seropositivity rates are lower in British patients

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with haemophilia B (Christmas disease) but cases of the Acquired Immunodeficiency Syndrome (AIDS) have been reported.⁴

The cutaneous manifestations of HIV infection have been mainly studied in homosexual men.^{5,6,7} One study has suggested that some dermatoses may be considered to be early warning signs for AIDS.⁶ No other groups at risk of HIV infection have been investigated for skin disease. We have, therefore, studied a group of haemophiliacs to determine whether or not the observations and conclusions made in sexually acquired HIV infection apply to non-homosexual patients with transfusion related disease.

METHODS

Forty-one male patients were studied, 40 with haemophilia A and one patient with haemophilia B (Christmas disease). The patients were attending the Oxford Haemophilia Centre for routine review.

None of the patients complained of any general symptoms and all were unaware of any skin disease. No patient had lymphadenopathy. Each patient was interviewed and examined by a dermatologist (NRT) who was unaware of the patient's HIV antibody status at the time of examination. Ethical approval was granted by the Central Oxford Research Ethics Committee.

Each patient completed a questionnaire giving details of previous medical and dermatological problems, personal and family history of atopic diseases, and past sun-exposure. They underwent a full dermatological examination.

A control population of 16 male patients was obtained from the dermatology pigmented lesion clinic. None of the control patients was a homosexual, haemophiliac, intravenous drug abuser or a sexual partner of these groups.

Statistical analysis was performed using the Chi-squared test with the Yates correction.

RESULTS

Twenty-four patients (58.5%) were seropositive for HIV antibody (age range 16–70, median 31.8). Seventeen patients (41.5%) were seronegative (age range 9–68, median 38.6), including one patient with Christmas disease. All 24 seropositive patients were asymptomatic and classed as grade II under the United States Centers for Disease Control (CDC) classification.⁸

The questionnaire data did not reveal any relationship between the presence of skin disease and previous medical or dermatological problems, family history of atopy, or past sun-exposure. One HIV antibody negative child had both a personal history and clinical signs of atopic dermatitis.

The variety of skin problems seen in the group are illustrated in Table 1. A difference in the prevalence of four conditions was seen when the HIV antibody positive group was compared with the HIV antibody negative group: 11 (27%) patients had seborrhoeic dermatitis—10 HIV antibody positive, a folliculitis mainly of the chest, face and back (but excluding the relatively common folliculitis of the thigh area seen frequently in healthy men) was seen in eight (20%) patients—six HIV antibody positive, four (10%) patients had candidiasis, three oropharyngeal and one intertriginous—all four were HIV antibody positive, and three (7%) patients had *Trichophyton rubrum* infection of the nails—all three were HIV antibody positive. None of these four dermatoses were seen in a group of 16 non-haemophiliac control patients.

TABLE 1. Dermatoses detected among 41 haemophiliacs divided into HIV antibody positive and negative groups

	Total	HIV + ve	HIV - ve
Seborrhoeic dermatitis	11	10	1
Folliculitis	8	6	2
Candidiasis	4	4	0
Onychomycosis	3	3	0
Keratosis pilaris	2	1	1
Acne vulgaris	2	1	1
Atopic eczema	1	0	1
Asteototic eczema	1	0	1
Hand eczema	1	0	1
Lichen planus	1	0	1
Actinic keratoses	1	0	1
Halo naevi	1	0	1

DISCUSSION

Seborrhoeic dermatitis,^{5,6,7,9} candidiasis,^{5,6,7,10} dermatophyte infection,^{5,6,7,11} and follicular inflammation^{5,6,7,12} are all described as cutaneous manifestations of HIV infection. This study shows an increased prevalence of these four dermatoses in HIV antibody positive compared with HIV antibody negative haemophiliacs. This difference has not previously been reported, and contrasts with the findings of previous studies in homosexual men with HIV infection.

The skin diseases seen in 172 homosexual men have been reported by Muhlemann *et al.*⁶ Three groups were described: AIDS, persistent generalized lymphadenopathy (PGL), and a 'high-risk' group. The last group was defined as male homosexuals with either multiple sexual encounters in the United States or known sexual contact with an AIDS patient. One hundred and forty-four asymptomatic male homosexuals (46 HIV antibody positive, 98 HIV antibody negative) served as controls. Three skin diseases were seen amongst the patients with AIDS, PGL and the high-risk group that were not seen in the control patients: chronic acneiform folliculitis, beard and neck impetigo, and extensive *Trichophyton rubrum* infection of the skin and nails. These conditions were regarded by the authors as early warning signs of AIDS.

Seborrhoeic dermatitis was seen in one of nine patients (11%) with AIDS, four of 15 (27%) with PGL, and none of the high-risk patients. The condition was also seen in four of 46 (9%) HIV antibody positive and three of 98 (3%) HIV antibody negative controls. Although seborrhoeic dermatitis was significantly more frequent in the PGL group when compared with controls, there was no significant difference in the prevalence of seborrhoeic dermatitis found between the two control groups, and this contrasts with the findings of our study, where the condition was seen in 42% of HIV antibody positive and 6% of HIV antibody negative haemophiliacs ($P < 0.05$).

An axillary and acneiform folliculitis was seen in the AIDS, PGL, and high-risk homosexual groups. Minor folliculitis was seen in one of 98 (1%) HIV antibody positive and none of the HIV antibody negative homosexual control groups. This compares with our finding of folliculitis in six of 24 (25%) HIV antibody positive and two of 17 (12%) HIV antibody negative haemophiliacs.

Mucocutaneous candidiasis was seen in one of 42 (2%) of the HIV antibody positive controls in the study by Muhlemann *et al.* This contrast with our finding of four cases in 24 (17%) HIV antibody positive haemophiliacs. None of the HIV antibody negative patients in either study had candidiasis.

Trichophyton rubrum infection was not seen in either of the homosexual control populations. It was present in three of 24 (13%) HIV antibody positive haemophiliacs.

The significance of these differences in the prevalence of certain skin diseases between asymptomatic HIV antibody positive and negative homosexual men and comparable but smaller groups of haemophiliacs is unclear. However, our study suggests that, when compared with homosexuals, certain HIV-associated dermatoses may be developing earlier in haemophiliacs. Such a difference between these two risk groups would be in keeping with other evidence to suggest that HIV infection might behave differently in its sexually acquired and transfusion acquired forms.

Other features of HIV infection that, from the literature, seem to be specific to haemophiliacs include an apparently long incubation period (defined as the time from seroconversion to the development of AIDS),¹³ and a low incidence of transmission of HIV infection to heterosexual partners,¹⁴ with few reported cases of AIDS¹⁵ and AIDS related complex (ARC).¹⁶ In addition, a postal survey of all haemophilia centres listed with the National Hemophilia Foundation in the United States suggested a low rate (0.6%) of HIV infection in children with haemophiliac fathers.¹⁷

There is also immunological and biochemical evidence to support the belief that the behaviour of HIV infection differs between haemophiliacs and other risk groups.^{18,19}

HIV related Kaposi's sarcoma (KS) and oral hairy leukoplakia are both conditions almost entirely confined to homosexual men, with no cases in non-homosexual haemophiliacs reported in the literature. The incidence of KS also appears to be extremely low in other risk groups including transfusion recipients and intravenous drug abusers.²⁰ It seems likely that other factors are important in the aetiology of these diseases in homosexual men, including cytomegalovirus²¹ or Epstein Barr virus infection.²²

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Double-blind, placebo-controlled study to evaluate the efficacy of fish oil and low-dose UVB in the treatment of psoriasis

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SUMMARY

Since eicosanoids have been implicated in the pathogenesis of psoriasis, less potent eicosanoid mediators derived from fish oil might improve psoriasis. Using a double-blind, randomized, parallel design, 18 patients with stable, plaque psoriasis received capsules of either fish oil or identical-appearing placebo olive oil for 15 weeks, with concomitant sub-erythral UVB in weeks 3 to 11. At the conclusion of phototherapy, and 4 weeks later, patients in the fish oil group had a greater decrease in the total body surface area of psoriasis and more improvement compared to patients in the olive oil group. The improvement in the fish oil group was statistically significantly greater for all parameters compared to the change in the olive oil group. The apparent safety and general health-promoting features of fish oil could provide an ideal adjunctive therapy for psoriasis.

The oils of cold water fish are rich in long-chain polyunsaturated fatty acids, in particular those of the omega-3 series, e.g., eicosapentaenoic acid (EPA, 20 carbons and 5 double bonds, 20:5) and docosahexaenoic acid (DCHA, 22 carbon atoms and 6 double bonds, 22:6). Omega-3 indicates that the first double bond of the fatty acid, counting from the methyl end, occurs at the third bond. The polyunsaturated acids of fish oil have biological effects that differ from those in vegetable oil, such as linoleic acid, which belong to the omega-6 series.

In involved plaques of psoriasis there is a marked increase in the levels of eicosanoids, e.g. arachidonic acid (AA), leukotriene B₄ and 12-hydroxyeicosatetraenoic acid (12-HETE).¹ In psoriasis, the 5-lipoxygenase pathway which converts AA to leukotrienes is enhanced and is associated with inflammation and cell proliferation. EPA can compete with AA as a substrate for

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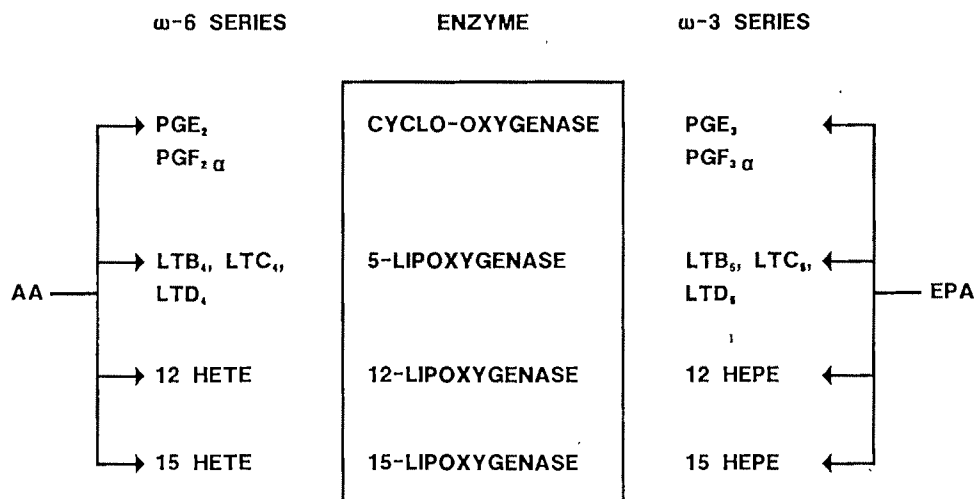


FIGURE 1. Diagram showing the metabolism of arachidonic acid and eicosapentaenoic acid (PG, prostaglandin; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; EPA, eicosapentaenoic acid; DCHA, docosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid).

cyclo-oxygenase and lipoxygenase with subsequent production of less biologically active metabolites,² such as leukotriene B₅ (Fig. 1). A diet rich in EPA would be expected to lead to an increased production of EPA metabolites of the omega-3 series, and a decrease in the metabolites of AA of the omega-6 series (Fig. 1). A reduction in the inflammatory and proliferative components of psoriasis could occur, if dietary fish oil were taken regularly.

Administration of fish oils, containing large amounts of omega-3 fatty acids, have been reported to be of benefit in the treatment of psoriasis³⁻⁸ and atopic dermatitis.⁹ We first demonstrated in an 8 week open trial that dietary fish oil supplementation with Max-EPA^R 60-75 gm per day (10.8-13.5 g of EPA) resulted in modest improvement of psoriasis.³ At similar doses of fish oil (9-12 g of EPA per day) for up to 3 months, other investigators noted clinical improvement in the majority of patients^{4,5} Bittiner *et al.*^{6,8} found fish oil (1.8 g EPA per day) to have some beneficial effect in psoriasis, whereas Bjorneboe *et al.*⁷ reported no such improvement using the same dose of fish oil.

The limited value of fish oil in all these studies suggests that it is not a monotherapy for psoriasis, but should be used as adjunctive therapy to more potent treatments. We decided to carry out a double-blind, randomized, placebo-controlled trial of parallel design to determine the efficacy of combined therapy with fish oil (3.6 gm EPA daily) and suberythral doses of ultraviolet B (UVB) phototherapy twice weekly in mild to moderate psoriasis.

METHODS

Twenty adult subjects with stable psoriasis vulgaris (10-50% total body surface area [TBSA] involvement) were chosen for this study. All patients had skin Type II or III and had no oral medications for psoriasis for the previous 4 weeks, and no topical therapy other than emollients for 2 weeks. During the course of the study, patients were permitted to use only the experimental therapy and emollients. Patients were asked to follow their normal diet during the

course of therapy. Patients with acute guttate psoriasis, and pregnant or lactating women were excluded. Informed consent was obtained from each patient and the protocol was approved by the review board of the institution.

The subjects were randomly assigned to two groups. One group received 10 fish oil capsules twice daily (for a daily total of 3.6 g of EPA and 2.4 g of DCHA; Max-EPA^R; R.P. Scherer, Troy, Michigan, U.S.A.). The other group was given identical appearing capsules containing olive oil (0 mg EPA and DCHA; 76% oleic acid, 9% palmitic acid, 7% linoleic acid, 2% stearic acid; R.P. Scherer, Troy, Michigan, U.S.A.). Nine subjects (six males and three females) with a mean age of 41 years (range 25–65) and mean TBSA involvement with psoriasis of $41 \pm 5\%$ received fish oil capsules. Eleven subjects (eight males and three females), with mean age of 51 years (range 22–74) and TBSA $34 \pm 3\%$ received placebo olive oil capsules. Patients were given fish oil (or placebo) for the first 3 weeks of the study (weeks 0–3). At this point concomitant twice weekly therapy with suberythral doses of UVB phototherapy for the next 8 weeks was initiated (weeks 3–11). For the last 4 weeks of the study (weeks 11–15), only fish or olive oil was given.

For each patient, prior to UVB phototherapy, the minimal erythema dose (MED) required to produce faint erythema was determined. Ultraviolet B therapy was delivered to all patients, utilizing an Ultralight^R phototherapy cabinet. Light therapy was initiated at 80% of the MED dose. In patients with skin type II, the UVB dosage in Joules was increased by a maximum of 10% just prior to the next phototherapy treatment if the patient had no UVB-induced erythema. In patients with skin type III, the corresponding increase was 20%. No increase in the UVB dosage was carried out if erythema was present when the patient reported for a UVB treatment.

Patients were seen on a weekly basis for the first 3 weeks, and then every 2 weeks for the remainder of the study. Compliance was determined by history and by asking the patients to bring with them the medication containers. The severity of psoriasis was determined by measuring the TBSA and using a grading scale that assessed each of the parameters of scale, erythema and thickness (0 = absent, 1 = trace, 2 = mild, 3 = mild-moderate, 4 = moderate, 5 = moderate-severe, 6 = severe).

Changes in scaling, erythema, thickness, TBSA and overall global response between the fish and olive oil groups were compared using the Students' two sample *t*-test. Differences between the two groups were assessed at pretherapy, the initiation of UVB therapy (week 3), the midpoint of UVB therapy (week 7), the conclusion of UVB therapy (week 11), and the end of the study (week 15; 4 weeks off UVB therapy).

Means are reported with a confidence interval of $\pm$ one standard error. All *P* values are two-sided.

RESULTS

Eighteen of 20 patients completed the 15 week study. One patient dropped out from each of the fish and the olive oil groups because of lack of response. Data is presented only for patients completing the study. None of the patients in either group reported any side-effects.

The scale, erythema, thickness and percentage TBSA during the study are shown in Table 1. The sum of the scale, erythema and thickness scores (0 = no disease present to 18 = severe disease) at pretherapy, weeks 3, 7, 11 and 15 are presented in Fig. 2. In both the fish and olive oil groups, the pretherapy severity of psoriasis was of a comparable nature.

In the group receiving fish oil there were four patients with each of skin types II and III; in the

TABLE 1. Psoriasis severity parameters for fish and olive oil groups

Parameter*		Week 0 Pretherapy	Week 3 Oil alone	Week 7 Oil + UVB	Week 11 Oil + UVB	P†	Week 15 Oil alone	P‡	Per cent change at end of study (Week 15) compared to pretherapy
Erythema	FO	3.5 ± 0.3	3.0 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	NS	2.0 ± 0.3	0.02	-43%
	OO	3.5 ± 0.3	3.2 ± 0.2	2.9 ± 0.3	3.0 ± 0.3		3.5 ± 0.5		0%
Thickness	FO	3.3 ± 0.6	3.2 ± 0.4	2.3 ± 0.4	2.0 ± 0.4	NS	1.7 ± 0.4	0.006	-48%
	OO	3.0 ± 0.2	3.0 ± 0.2	2.4 ± 0.3	2.5 ± 0.3		3.2 ± 0.2		7%
Scale	FO	3.1 ± 0.4	2.7 ± 0.5	1.2 ± 0.7	1.6 ± 0.6	NS	2.0 ± 0.7	0.008	-35%
	OO	3.3 ± 0.2	3.1 ± 0.4	2.7 ± 0.3	2.4 ± 0.3		3.4 ± 0.3		+9%
TBSA%	FO	41 ± 5	35 ± 4	32 ± 4	27 ± 4	0.05	22 ± 5	0.0001	-46%
	OO	34 ± 3	36 ± 3	36 ± 3	34 ± 5		45 ± 2		+32%

* Mean ± SE, based on 6 point scale for erythema, thickness, scale; TBSA = % total body surface area involved with psoriasis.

FO = Fish Oil, n = 8; OO = Olive Oil, n = 10.

† By Student's two-sample *t*-test comparing the mean changes for each group from pretherapy to week 11.

‡ By Student's two-sample *t*-test comparing the mean changes for each group from pretherapy to week 15.

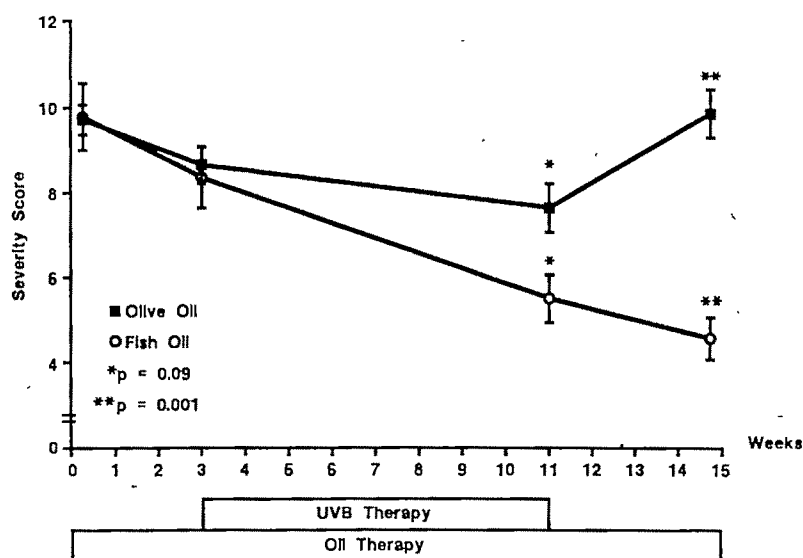


FIGURE 2. Mean sum of scores for scale, erythema, thickness at pretherapy, week 3, week 11 and week 15 in the fish and olive oil groups. Severity score: 0 = no disease and 18 = severe disease; the bars indicate standard error. *P* values were determined by Student's *t*-test comparing the mean sum of scores between the fish and olive oil groups.

olive oil group, there were five patients with each of skin types II and III. Between weeks 3 and 11, the mean total amount of UVB received by the fish and olive oil groups was 1.6 ± 0.2 Joules and 1.4 ± 0.3 Joules, respectively ($P = \text{NS}$).

At the end of week 3, the group receiving fish oil appeared to be improving more than the olive oil group (Table 1). At week 11 the group receiving fish oil had improved to a greater extent compared to pretherapy, than the patients receiving olive oil ($P = 0.05$ for TBSA) (Table 1). At week 15, the fish oil group had a 35% decrease in scale compared to pretherapy, while the group receiving olive oil had a 9% increase; the comparable figures for erythema, thickness and TBSA were -43% vs. 0%, -48% vs. +7% and -46% vs. 32% respectively. For both the fish and olive oil groups, the mean change in the scale, erythema, thickness and TBSA scores between end of therapy (week 15) and pretherapy were calculated and the fish oil group had a statistically significantly better response for all parameters (Table 1, Figure 2).

DISCUSSION

In our experience,³ fish oil, even at high doses, is not a practical monotherapy for psoriasis. This has also been reported in other studies in which psoriasis demonstrated only mild to moderate improvement^{4,5,6,8} or no improvement.⁷ In this study, there was no statistical advantage of fish oil over olive oil therapy in the first 3 weeks when oil therapy alone was used. After an additional 8 weeks of oil therapy combined with UVB treatment, we determined that the group receiving fish oil had a decrease in total body surface area, while the group receiving olive oil had no change in this parameter ($P = 0.05$) (Table 1). After UV therapy had been stopped and oil therapy alone continued for 4 weeks, the fish oil group improved in all parameters studied compared to pretherapy while the group receiving olive oil had not changed or had worsened. The improvement in the fish oil group was statistically significantly greater for all parameters compared to the change in the olive oil group. These data show that improvement caused by fish oil and low dose UVB at week 11 was maintained for a further 4 weeks by fish oil therapy but not by olive oil administration.

Twice weekly suberythral UVB for 8 weeks is generally not sufficient as a monotherapy to clear psoriasis. Our UVB protocol was designed to be only minimally effective so as to maximize the relative effect of fish oil. With our study design, it is difficult to accurately determine the contribution of UVB relative to fish oil therapy toward the improvement in psoriasis. However, the degree of improvement noted in the group receiving low dose UVB and fish oil therapy was greater than would be expected following monotherapy with fish oil.

At the doses of fish oil (10.8–13.5 gm of EPA) used in our first study,³ no adverse side-effects were noted other than occasional 'fishy taste' on the breath upon eructation. In the present study, fish oil and placebo were given in identical-appearing tasteless, gelatinous capsules. In this dose and form, patients noted no 'fishy taste' on the breath upon eructation. Other studies,^{4–8} using similar or lower doses of fish oil for the treatment of psoriasis, have not reported side-effects. However, Saynor *et al.*¹⁰ found that patients who took 3.6 g of EPA daily for 2 years had significantly elevated bleeding times compared to those patients receiving 1.8 g of EPA per day. The clinical significance of this is not clear, since in one study the effect on platelets in patients taking 10 g of EPA per day for 1 month was less than occurs with one aspirin tablet (325 mg) taken daily.¹¹ It may be advisable not to prescribe fish oil to patients with bleeding disorders who are at risk for haemorrhagic complications.

Therapy with fish oil has other advantages besides its value in the treatment of the skin disease.¹² In both normal and hyperlipidaemic subjects, dietary supplementation with fish oil

rich in EPA and DCHA may result in reduction of serum triglycerides, cholesterol, and cholesterol-rich low density lipoproteins (LDL),^{13,14} and an elevation of serum high density cholesterol.^{15,16} Marsden^{17,18} and Lowe *et al.*¹⁹ have shown that supplementation of diet with Max-EPA^R reduces hyperlipidaemia induced by isotretinoin and etretinate. Thus the addition of fish oil to retinoid therapy for psoriasis may lead to a more desirable lipid profile, plus a modestly added therapeutic benefit. Some studies have reported that supplementation of the diet with fish oil may result in a reduction of platelet aggregation, blood viscosity and thrombotic tendency.²⁰⁻²² Fish oil in the diet may also exert other cardioprotective effects. Kromhout *et al.*²³ studied fish consumption in 852 middle aged men without coronary heart disease in the Netherlands. They were followed longitudinally for 20 years. Mortality from coronary heart disease was more than 50% lower among those who consumed at least 30 g of fish per day compared to those who did not eat fish. Omega-3 fatty acids have also been reported to reduce blood pressure.¹²

Since fish oil therapy is practical, has certain other potential advantages and a relative lack of side-effects, combination therapy of UVB and fish oil should be considered in the treatment of psoriasis. Fish oil administration may also provide a therapeutic advantage when combined with other therapies for psoriasis. Further studies need to be carried out to determine whether combined therapy with fish oil can lengthen the period of remission in patients with psoriasis.

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The short-term benefit and long-term failure of ultraviolet light in the treatment of venous leg ulcers

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SUMMARY

Ultraviolet light has in the past been advocated for the treatment of venous leg ulcers on the assumption that it increases skin blood flow and reduces skin hypoxia. Our results show that UV light increases skin-oxygen tension of the lower leg and inhibits the normal vasoconstrictor response on standing. However, this effect is short lived and is followed after 2 days by a return of the vasoconstrictor reflex and a marked decrease in skin oxygenation which continues for at least 2 weeks. Since UV irradiation improves skin oxygenation for only 48 h, it cannot be recommended as a form of treatment for venous leg ulcers.

It has been suggested that ultraviolet light is of value in the treatment of venous leg ulcers because it stimulates skin blood flow and reduces skin hypoxia,¹ and yet earlier studies have shown increased blood flow in legs affected by venous ulceration.^{2,3} Various theories have been put forward to explain this paradox. Arteriovenous shunting was suggested,⁴ and has gained some support from studies showing premature venous filling on dynamic angiography of affected limbs.^{5,6} However, some of these studies have used special measures to produce vasodilation and have been deficient in suitable control subjects on ethical grounds.⁶ Attempts to demonstrate the presence of shunts by direct techniques such as labelled microspheres have invariably failed.^{7,8} More recently, it has been proposed that skin hypoxia arises from impairment of oxygen diffusion from the capillaries to the skin surface as a result of low permeability of pericapillary fibrin deposits.⁹ However, there is no direct evidence for this and skin oxygen tension measured at 37°C in recumbent patients is increased compared to levels found in normal subjects.¹⁰

We have recently shown that standing leads to cutaneous hypoxia¹⁰ which is considered to be the result of reflex arteriolar vasoconstriction in response to increased venous pressure.^{11,12} Such lowering of skin oxygen tension leads to impairment of aerobic respiration. During exercise in normal subjects, venous pressure falls¹³ and skin oxygen tension rises.¹⁰ In patients with venous incompetence, venous pressure remains high during exercise¹⁴ and there is no rise in oxygen tension.¹⁰ Thus, exercise in normal subjects removes the stimulus,¹² i.e. raised

venous pressure, which leads to hypoxia. We have concluded that the cause of venous leg ulcers is prolonged hypoxia induced by reflex vasoconstriction.

Improvement of skin oxygenation can be achieved by restoring the fall in venous pressure during exercise by surgery or by elastic compression.¹⁵ Skin oxygenation may also be improved by inhibition of the normal reflex arteriolar vasoconstriction in response to raised venous pressure. Although this could be achieved by systemic vasodilator therapy, such treatment would cause postural hypotension. Local inhibition of the vasoconstrictor reflex would avoid this unacceptable side effect. Ultraviolet light is well known to produce erythema and increased skin blood flow. This report describes the effect of ultraviolet light on skin oxygen tension of the lower leg and the changes in vascular responses during the first 15 days after irradiation.

METHODS

Three male and three female control subjects (23–44 years) and six female patients (57–77 years) with recently healed chronic venous leg ulcers were studied. Simultaneous measurements of $tcPo_2$ were obtained using two transcutaneous polarographic oxygen electrodes (Hellige Oxymonitor).

A circular area of skin 2 cm in diameter was irradiated with three minimal erythema doses (3 MED) using a water cooled high pressure mercury vapour UV lamp (Kromayer). The electrodes were placed on the anterolateral surface of the lower leg 20 cm above the sole, 5 cm apart. The temperature of the electrodes was initially thermostatically controlled at 37°C and subsequently at 44°C. One electrode was placed over the UV-irradiated area and the second on an adjacent untreated control site. The oxygen tensions of the treated and control area were measured at various intervals between 6 h and 15 days after irradiation. Subjects were kept recumbent in a quiet room at a temperature of 23–25°C. The initial electrode temperature was 37°C and a steady reading was reached after 20–30 min. Measurements were recorded and the subjects then stood upright for 5 min using the edge of a couch as a support to minimize leg muscle activity and the new stable values were noted. The subjects were next asked to stand on one leg and the leg under investigation was plantar- and dorsi-flexed at the rate of 60 times per min for 2 min. This rate was maintained with the help of a metronome. $TcPo_2$ was recorded at the end of this standardized exercise test. Steady-state $tcPo_2$ values were noted when the subjects were again standing motionless after exercise and later during recumbency. Finally, the response to local heating was measured by raising the temperature of the electrodes from 37°C to 44°C. The new steady-state values in recumbent subjects were recorded 20–30 min after increasing the electrode temperature.

RESULTS

In the first 48 h after exposure (Table 1), $tcPo_2$ measured at 37°C in the recumbent position is increased at the UV exposed site ($P=1.9 \times 10^{-6}$) compared to the untreated control. The normal fall in $tcPo_2$ on standing (column 2) is inhibited by UV light. Both these effects are seen in patients and in controls. Figure 1 shows simultaneous tracing from a UV-irradiated site 24 h after exposure and from an adjacent unirradiated control area. In the control area, there is a fall in $tcPo_2$ on standing and an increase on exercise, whereas $tcPo_2$ remains elevated at the UV exposed site during standing and exercise.

The values of control and irradiated sites in recumbent controls and patients during the period of 2–15 days after UV exposure are shown in Table 2. The values at the irradiated sites

TABLE 1. Initial effect of UV on posturally-induced changes to tcPO₂ of the leg

	Control site		UV irradiated site	
	Recumbent tcPO ₂ (mm Hg)	Standing* tcPO ₂ (mm Hg)	Recumbent tcPO ₂ (mm Hg)	Standing† tcPO ₂ (mm Hg)
Normal control subjects	1	0	36	31
	1	0	30	38
	1	2	22	23
	2	0	41	46
	3	0	29	13
	3	0	23	18
	5	1	13	19
	6	2	25	28
	7	1	19	18
	7	0	13	16
	9	0	16	6
	10	0	36	40
	13	1	36	35
	19	4	36	42
Median	5.6	0.4	27.0	25.2
Venous ulcer patients	1	0	49	56
	2	0	55	64
	11	0	27	5
	20	7	43	44
	28	4	64	52
	30	9	64	73
Median	15.8	2.3	52.0	53.7

TcPO₂ (mm Hg) measured at 37°C over UV exposed sites 6-48 h following irradiation and simultaneous tcPO₂ values on adjacent control sites during recumbency and on standing.

* Response to standing on control sites.

Recumbent tcPO₂ > standing tcPO₂ (n = 19).

Recumbent tcPO₂ < standing tcPO₂ (n = 1).

2-tail-test based on binomial expansion, $P = 2.1 \times 10^{-5}$.

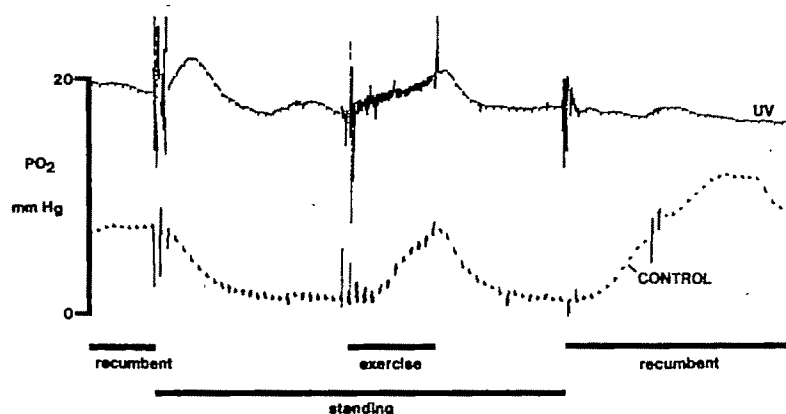
† Response to standing following UV exposure.

Recumbent tcPO₂ > standing tcPO₂ (n = 8).

Recumbent tcPO₂ < standing tcPO₂ (n = 12).

2-tail-test based on binomial expansion, $P = 0.49$.

Spearman rank correlation, UV recumbent vs. UV standing (control subjects), $R_s = 0.80$, $P < 0.001$. This result, together with that above suggests that the inhibition of the response to standing is independent of the degree of erythema (and consequent elevation of tcPO₂) induced by UV irradiation following exposure to a dose of 3 MED. Limited observations show that the fall in tcPO₂ is only partially inhibited by 2 MED even when recumbent tcPO₂ values are similar to those seen after exposure to 3 MED.



responses 24 hours after irradiation

FIGURE 1. Simultaneous recordings of skin oxygen tension measured at 37°C from a normal control site and from an adjacent site exposed to UV (3 MED) 24 h previously. Note increased tcPO₂ of the exposed site and inhibition of the fall of tcPO₂ on standing.

demonstrated a marked reduction in skin oxygen during this period in both groups. In Figure 2, the ratios recumbent tcPO₂ irradiated/recumbent tcPO₂ control are plotted against time. In both patients and controls the ratios fall in the first 48 h and thereafter remain below unity for the duration of the study period. Table 3 shows the effect of exercise in control subjects. The rise in tcPO₂ on exercise is diminished in irradiated sites 2–15 days after exposure compared with unirradiated control areas. As none of the ulcer patients showed any increase in oxygen tension in unirradiated skin during exercise, the effect of UV irradiation on this response could not be demonstrated. The reduction in the level of oxygen tension at the irradiated site during recumbency and exercise 13 days after exposure is clearly seen in Figure 3 when compared with the control.

Using the Spearman rank correlation test on data in Tables 2 and 3, there is close correlation between tcPO₂ of the paired simultaneous values from UV exposed and control sites (Table 2, $R_s = 0.70$, $P < 0.01$ for control subjects, $R_s = 0.60$, $P > 0.05$ for ulcer patients and $R_s = 0.47$, $P < 0.05$ overall; Table 3, $R_s = 0.75$, $P < 0.001$). These correlation coefficients show that there is a close relationship between the values obtained from adjacent paired electrodes at 37°C and that the reduction of skin oxygen tension by irradiation is a consistent phenomenon independent of normal variation in blood flow.

Table 4 shows that the rise in tcPO₂ following local heating (induced by raising the electrode temperature to 44°C) is diminished in the irradiated site 2–15 days after exposure in both patients and control subjects ($P = 7.6 \times 10^{-6}$).

Preliminary investigations to select the exposure level to be used throughout the study showed that doses of less than 3 MED did not completely inhibit the fall in tcPO₂ on standing 24 h after exposure whilst greater exposure prolonged the duration of the initial elevation of tcPO₂.

TABLE 2. Late-onset reduction in skin oxygen tension following UV irradiation

	Control site (A) tcPO ₂ (mm Hg)	UV irradiated site* (B) tcPO ₂ (mm Hg)	Ratio (B/A)
Normal control subjects	1	0	<0.1
	2	1	0.28
	2	1	0.53
	2	2	1.03
	2	1	0.26
	5	4	0.88
	6	5	0.82
	7	10	1.47
	10	5	0.47
	10	3	0.30
	11	9	0.85
	12	3	0.29
	13	2	0.15
	14	10	0.75
	14	10	0.75
	16	6	0.37
	21	8	0.37
Median	9.9	4.2	0.47
Venous ulcer patients	10	3	0.29
	15	2	0.13
	19	1	0.06
	21	6	0.31
	25	6	0.22
Median	19.0	2.9	0.22

TcPO₂ (mm Hg) measured at 37°C in recumbent subjects on sites irradiated with UV 2–15 days previously, simultaneous tcPO₂ values measured on adjacent unirradiated skin and the ratios of these paired values. The ratios were calculated from original data, whereas tcPO₂ values in columns A and B are rounded to the nearest whole number.

* tcPO₂, UV > tcPO₂, Control (*n* = 2).

tcPO₂, UV < tcPO₂, Control (*n* = 20).

2-tail-test based on binomial expansion, $P = 1.1 \times 10^{-4}$.

Spearman rank correlation, control *vs.* UV (control subjects), $R_s = 0.70$, $P < 0.01$

Spearman rank correlation, control *vs.* UV (ulcer patients), $R_s = 0.60$, $P > 0.05$

DISCUSSION

The results show that UV irradiation alters skin blood flow and its regulation. The effects of UV irradiation are qualitatively similar in ulcer patients and normal control subjects. During the first 48 h after UV exposure there is an increase in skin PO₂ of the lower leg in both normal and ulcer subjects. This is the result of increased skin blood flow and correlates with observations

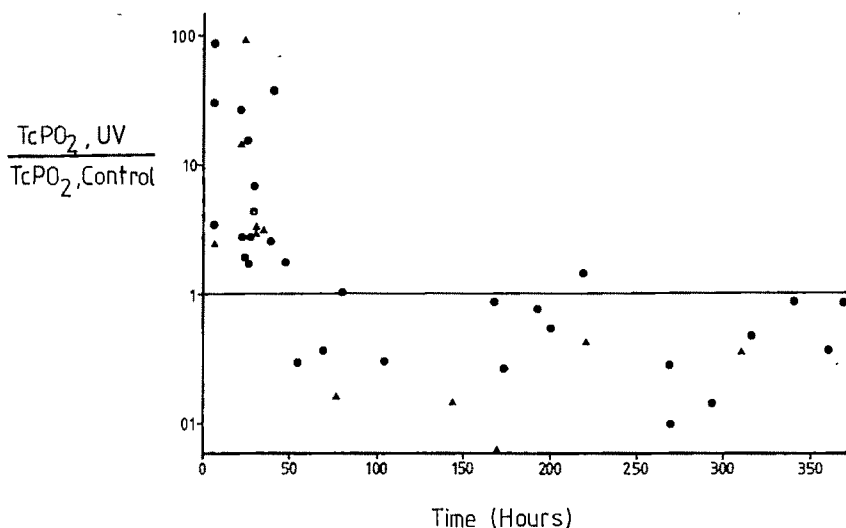


FIGURE 2. Ratio of control $tcPO_2$:UV-exposed $tcPO_2$ measured at an electrode temperature of $37^\circ C$ in recumbent subjects plotted against time. $TcPO_2$ is greater in the UV-exposed skin during the first 48 h and is thereafter lower than that of the paired control sites. No difference between the normal control subjects' and the leg ulcer patients' values are apparent. ● = Control subjects; ▲ = Venous ulcer patients.

that have shown increased prostaglandin^{16,17} and histamine levels^{17,18} in the skin during the first 48 h after UV irradiation. The highest levels are reached about 6 h following irradiation and return to normal after 24–48 h.^{16,17}

No significant change in $tcPO_2$ occurs on standing during the first 48 h after UV exposure and the $tcPO_2$ of irradiated skin remains elevated. In contrast, adjacent non-irradiated skin shows the normal vasoconstrictor response on standing, as shown by a fall in $tcPO_2$. The increased skin blood flow in the recumbent position and the inhibition of the vasoconstrictor response on standing shows that UV light prevents cutaneous hypoxia during the first 48 h. Such an effect can be expected to be beneficial in the treatment of venous leg ulcers.

Although levels of mediators have returned to normal by 48 h, UV erythema persists. $TcPO_2$ in irradiated areas measured more than 48 h following exposure was less than that of adjacent control skin. The delayed reduction in skin oxygen tension was seen in both leg ulcer patients and normal subjects and persisted until the end of the study at 15 days. We have also demonstrated that the vasodilator responses to exercise and local heating are diminished in the irradiated skin during the period 48 h to 15 days after exposure.

There is significant correlation between the paired irradiated and control values (Tables 2, 3 and 4), despite the different test conditions on the adjacent sites. Because there is no significant correlation between the control values and the paired ratios (Table 4), one may conclude that there is likely to be a simple linear relationship between the paired values used to calculate the ratios.

Lewis¹⁹ demonstrated that the flare in response to stimuli such as heating, freezing and histamine is reduced in UV irradiated skin and this has been confirmed by others.^{20,21} We have demonstrated a decreased flare in response to histamine and heating (unpublished observations). Furthermore, our results (Table 4) show that blood flow at the actual point of heating, as well as in the surrounding flare, is diminished by prior UV exposure. The flare induced by

TABLE 3. Diminished effect of exercise tcPO₂ in previously irradiated skin

	Control site (A) tcPO ₂ (mm Hg)	UV irradiated site* (B) tcPO ₂ (mm Hg)	Ratio (B/A)
	1	0	<0.1
	1	2	1.47
	2	1	0.36
	2	1	1.51
	3	2	0.65
	3	2	0.48
	3	2	0.71
	4	1	0.30
	6	0	0.07
	6	2	0.31
	8	1	0.14
	11	5	0.45
	11	3	0.26
	12	4	0.31
	15	5	0.33
	22	13	0.57
	24	11	0.47
Median	6.2	2.0	0.36

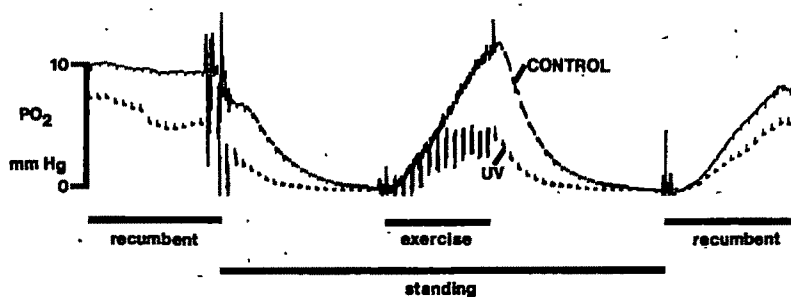
Simultaneous tcPO₂ (mm Hg) measurements at 37°C on sites irradiated 2-15 days previously and on adjacent control sites at the end of a 2 min exercise period in the standing position and the ratios of these paired values.

* tcPO₂, UV > tcPO₂, Control (n=1).

tcPO₂, UV < tcPO₂, Control (n=16).

2-tail-test on basis of binomial expansion, $P=2.7 \times 10^{-4}$.

Spearman rank correlation, control *vs.* UV; $R_s=0.75$, $P<0.01$.



responses 13 days after irradiation

FIGURE 3. Simultaneous recordings of skin oxygen tension measured at 37°C from a normal control site and from an adjacent site exposed to UV (3 MED) 13 days previously. TcPO₂ of the UV-exposed site is lower than that of the control, but the normal regulatory responses are apparent even though the overall tcPO₂ is diminished.

TABLE 4. UV-induced reduction in tcPO₂ in heated skin

	Control site (A) tcPO ₂ (mm Hg)	UV irradiated site* (B) tcPO ₂ (mm Hg)	Ratio (B/A)
Normal control subjects	43	23	0.54
	46	34	0.73
	52	15	0.29
	55	11	0.21
	56	43	0.77
	56	28	0.49
	56	42	0.75
	57	36	0.63
	58	32	0.54
	59	42	0.72
	61	38	0.61
	62	48	0.78
	67	47	0.70
Median	56	36	0.63
Venous ulcer patients	47	11	0.24
	51	22	0.46
	68	52	0.76
	72	32	0.44
	74	8	0.11
Median	68	22	0.44

TcPO₂ (mm Hg) measured at 44°C in the recumbent position on sites irradiated with UV 2–15 days previously, simultaneous tcPO₂ values of adjacent control sites and the ratios of these paired values.

* tcPO₂, UV > tcPO₂, Control (*n* = 0).

tcPO₂, UV < tcPO₂, Control (*n* = 18).

2-tail-test on basis of binomial expansion, $P = 7.6 \times 10^{-6}$.

Spearman rank correlation, control *vs.* UV, $R_s = 0.80$, $P < 0.01$.

Spearman rank correlation, control *vs.* ratio, $R_s = 0.24$, $P > 0.05$.

chemical and physical stimuli is mediated by an antidromic axon reflex. The diminished flare response in UV irradiated skin suggests that UV disrupts the axon reflex. Degenerative changes in Schwann cells, perineural cells and axoplasmic organelles have been demonstrated following UV irradiation.²² Observations of the time course of these changes have shown that they are most marked 48 h after exposure. Such structural changes following UV explains the observed alterations in reflex control of skin blood flow. The slow recovery from this UV induced damage to neural tissue would account for the prolonged time course of the second phase of the vascular response to UV light which we have observed.

Although the antidromic axon reflex vasodilation is impaired by UV light for at least 15 days, we have demonstrated that the vasoconstrictor reflex response to increased venous pressure on standing is restored as early as 48 h after exposure. This vasoconstrictor response involves stretch receptors in the deep veins and it is a spinal reflex.¹² UV irradiation is strongly absorbed by the skin so that the UV dose reaching the deeper components of the reflex is physiologically

insignificant. Our results show that there is no long term impairment of the efferent vasoconstrictor limb of this reflex by UV light.

UV exposure leads to a sequence of effects on skin blood flow and oxygen tension of the lower leg which may be summarized as follows. During the first 48 h there is vasodilation, increased skin oxygen tension and an inhibition of vasoconstrictor responses which corresponds to the time when vasodilator mediators are at their peak. After this there is a prolonged reduction of skin blood flow when the vasodilator response to heat is impaired and the vasoconstrictor reflex in response to venous distension has returned, leading to a marked reduction in $tcPO_2$ during recumbency and exercise. This prolonged effect may be explained by the observed damage to neurones and their supportive cells caused by UV light.

As the increased skin blood flow after UV irradiation lasts only 48 h and it is followed by a prolonged reduction in blood flow and a lowering of oxygen tension, our results provide no rational basis for using UV irradiation in the treatment of venous leg ulcers. However, there remains the possibility that UV light may have some other beneficial effect on venous leg ulcers.

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The treatment of decorative tattoos with the carbon dioxide laser

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SUMMARY

The carbon dioxide laser was used in the treatment of 51 patients with decorative tattoos. Sixty-eight treatments were performed under local anaesthesia. Morbidity from treatment was low. Results 6 months after treatment in 44 patients revealed excellent or good results in 24 patients and hypertrophic scarring in three patients. These results confirm the value of this laser in the treatment of tattoos and demonstrate the advantages of this therapy over other treatments currently employed.

Cutaneous decorative tattooing is a long established practice within society. The tattoo is often applied in teenage years and may become a source of regret and a social burden in later life. Consequently a number of methods for removal of the tattoo have been devised, including dermabrasion, salabrasion, cryotherapy and excision. Apart from surgical excision, where a skin graft may be required, the other methods suffer from the disadvantages of incomplete pigment removal, inability to control depth of destruction, necessity for repeated procedures, pain and unsatisfactory scarring. In theory, laser therapy of tattoos may avoid many of these disadvantages, and there are a number of reports of the treatment of tattoos with lasers.^{1–3} Although satisfactory results are generally reported, one group³ have abandoned laser therapy for surgical excision because of the high incidence of unsatisfactory scarring in the treatment of polychromatic professional tattoos in a pilot study.

The carbon dioxide laser emits infra-red laser light at a wavelength of 10,600 nm which is uniformly absorbed in water with minimal thermal transmission and scatter.⁴ This radiation is absorbed within the first 0.2 mm of tissue which is vaporized at 100°C. The defocused beam with a diameter of 3–5 mm may be used for controlled tissue vapourization, whilst the focused beam may be used for cutting tissue. There is wide experience in the use of the carbon dioxide laser in dermatology for a variety of conditions.^{5–9}

The advantages of using the carbon dioxide laser in the treatment of tattoos are its high

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precision, minimal unwanted tissue damage and possible reduced pain from the procedure. We present the results of a large series of patients with tattoos treated by the carbon dioxide laser.

METHODS

The details of the patients treated are shown in Table 1. It can be seen that far fewer women had professional tattoos. All patients were interviewed prior to treatment and those with a history of hypertrophic or keloidal scarring were excluded. The patients were advised of the high risk of a scar replacing their tattoo after treatment, and informed consent for treatment was obtained. Treatment details are shown in Table 2. All treatments were performed under local anaesthesia using a Coherent 451 Carbon Dioxide laser with a dermatological handpiece. Initially, using a defocused beam of 3 mm diameter and power output of 10–20 W (power density 150–300 W/cm²) in continuous mode, an airbrush technique was used to produce thermal blistering of the skin. The blister roof was examined histologically in five patients and was shown to consist of partial or full thickness epidermis with prominent follicular plugging (Fig. 1).

Removal of the epidermis revealed the tattoo more vividly and further passes of the laser were performed to vapourize progressively the tattoo pigment. With about 90% of the tattoo removed by this technique, the laser handpiece was brought close to the wound to produce a focused beam with a diameter of 0.2 mm. Residual pigment was treated at 5–10 W with a pulse duration of 0.05 s (power density: 16,500–33,000 W/cm², energy fluence: 800–1600 J/cm²). This was performed in an attempt to minimize unwanted thermal damage to deeper structures.¹⁰ A subtotal clearance of the pigment was performed, leaving a minimal amount of more deeply situated pigment behind. This was to minimize damage to surrounding tissue, and because further pigment loss occurs in the inflammatory exudate that develops after treatment. In addition, the optical properties of the healed tissue will usually obscure most residual pigment.

The wounds were dressed with paraffin gauze and changed daily to encourage exudation. A pressure bandage was applied to sites at risk of keloidal scarring. All patients were reviewed

TABLE 1. Details of patients treated

51 Patients	Mean age 27.2 years (Range 18–51)	
30 males:	16 professional	14 amateur tattoos
21 females:	5 professional	16 amateur tattoos

TABLE 2. Treatment details

68 treatments:	1 treatment	37 patients
	2 treatments	11 patients
	3 treatments	3 patients
Sites treated:	Face 1	Forearm 20
	Knees 1	Upper arm/shoulder 17
	Wrist 1	Fingers/hand 9
		Neck 2

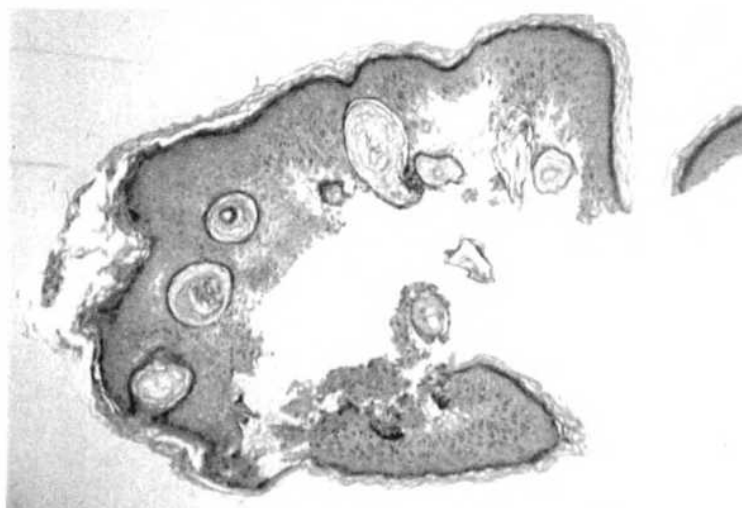


FIGURE 1. Photomicrograph of epidermal blister roof raised by carbon dioxide laser.

1 week after treatment and oral antibiotics administered if there was clinical evidence of spreading infection. Although most wounds showed slough and a purulent exudate at 1 week after treatment, adjacent skin in most cases looked uninvolved.

To assess laser wound infection rates, routine wound swabs were sent for bacteriological culture in 10 successive patients immediately after laser therapy before the dressings were applied. In these, and a further 10 successive patients, a similar swab was sent for culture from the wound 1 week after laser treatment and results compared.

Assessments of the response to laser therapy were made 6 months after treatment. An excellent result was defined as a complete clearance of tattoo pigment with a flat scar which was normal skin colour or hypopigmented. A good result again showed complete clearance of tattoo pigment with a flat scar which was more noticeable by being red or hyperpigmented. As most patients were concerned that no evidence of their tattoo should remain, no cases with residual pigmentation were described as good results, although it will be possible to retreat these patients to eradicate visible pigment. Thus, a fair result was defined as a flat scar with residual pigment or a complete clearance of tattoo pigment with an irregularly raised scar. Any hypertrophic scarring was recorded as a poor result.

RESULTS

The overall results 6 months following laser therapy are available in 44 patients and shown in Table 3. An excellent or good response was seen in 24 of 36 patients seen at follow-up after 6 months. The excellent responses occurred in five amateur and five professional tattoos. Five were on the forearm, three on the shoulder and one each on the face and hand. Examples of satisfactory results are illustrated in Figures 2-5. The three poor results were hypertrophic scars, two occurring on the shoulder in one amateur and one professional tattoo, and one occurring in a professional tattoo on the forearm.

There was minimal morbidity from therapy, discomfort was mild, and no patient lost time from work. Three patients required systemic antibiotics for clinical wound infections.

As expected, the wound immediately after treatment was sterile in all but three patients,

TABLE 3. Results of treatment to tattoos

44 Patients	No.	(%)
Excellent	10	(22.7)
Good	14	(31.8)
Fair	9	(20.4)
Poor	3	(6.8)
Failed to attend	8	(18.2)



FIGURE 2. Excellent result after partial treatment to professional tattoo on forearm.



FIGURE 3. Excellent result after treatment to an amateur tattoo on forearm.



FIGURE 4. Good result after partial treatment to tattoo on shoulder.



FIGURE 5. Fair result to professional tattoo on neck showing residual pigment.

where only *Staphylococcus epidermidis* was grown. However, as can be seen from Table 4, by 1 week after treatment 50% of the wounds grew pathogens. However, within this group of 20 patients, the presence or absence of pathogens was unrelated to eventual outcome of treatment.

Of the 36 patients reviewed 6 months after laser therapy, 34 were pleased with the results and this included one patient with a hypertrophic scar.

TABLE 4. Wound infection rates 1 week after laser therapy

No. = 20		
Sterile 9	<i>S. aureus</i>	7
	<i>Gp. G. haem. strep.</i>	1
	<i>E. coli</i>	1
	<i>Coliforms</i>	1
	<i>S. epidermidis</i>	1

DISCUSSION

The result of this study of 51 patients with tattoos treated with the carbon dioxide laser has demonstrated that this laser is quick and easy to use and associated with a low incidence of side effects. A good or excellent result was achieved in two thirds of patients reviewed with an 8.5% incidence of hypertrophic scarring. However, 34 of 36 patients were pleased with the results and this reflects the desire of patients to be rid of their tattoo at any cost. Eighteen per cent of patients repeatedly failed to attend and these patients may all have had unsatisfactory results. Whilst this is possible, it is more likely these patients were part of the general trend of poor attendance of this group, particularly when all treatments were completed.

In contrast to James *et al.*,³ we were able to leave some pigment in the wound after treatment which was removed in the inflammatory exudate with daily dressings. This method, and the use of high power densities of short pulse duration may have contributed to the low incidence of hypertrophic scarring in our patients. However, two of 17 patients with tattoos on the shoulder and upper arm developed hypertrophic scars after treatment and particular care should be exercised in treating tattoos in this area. The high incidence of wound colonization by pathogens within 1 week of treatment militates against the early use of steroids in the prevention of hypertrophic scarring.

In comparison to other destructive techniques, carbon dioxide laser therapy has low morbidity and needs only one treatment session in the majority of cases. In particular, patients who had experienced salabrasion complained of significantly more pain from this technique compared with laser treatment. In comparison with surgical excision, no skin grafting is required to cover the defect, but there is a higher risk of scarring, and choice of therapy may depend on local availability.

In conclusion, the results of this study have demonstrated that the carbon dioxide laser is a suitable treatment for both amateur and professional decorative tattoos with a low incidence of side-effects and a high rate of patient satisfaction. With the techniques used, over two-thirds of patients had an excellent or good outcome from treatment with an 8.5% incidence of scarring. Particular care should be observed in the treatment of tattoos at sites at risk of keloidal or hypertrophic scarring.

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Darier's disease with peri-follicular depigmentation

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SUMMARY

We report a case of Darier's disease presenting with widespread peri-follicular depigmentation. Histology revealed the features of Darier's disease in the depigmented macules and an absence of melanocytes.

We report a very unusual variant of Darier's disease, presenting with widespread peri-follicular macular depigmentation. This depigmentation is a recognized, though an exceedingly rare feature of Darier's disease^{1–3} and has not previously been described in such a marked peri-follicular distribution.

CASE REPORT

The patient, a 28-year-old Asian warehouseman, presented 10 years ago with widespread macular depigmentation in an almost exclusively peri-follicular pattern most prominent on the trunk but also involving the proximal parts of the limbs (Figs 1 and 2). The macules ranged from 1–4 mm in diameter and were sometimes confluent. This has not changed in appearance over the last 10 years. Examination also revealed classical features of Darier's disease. He has brittle nails with red and white bands and V-shaped notches, pitting of the palms, fingertips and soles and warty lesions most prominent on the limbs (Fig. 3). At no time have any clinical features of Darier's disease apart from depigmentation been present on the trunk.

The histology of areas of depigmentation showed the characteristic features of Darier's disease with dyskeratosis, corps ronds and supra-basal clefts containing acantholytic cells (Fig. 4). Pigmentation was markedly reduced in the areas of Darier's activity and also in some adjacent areas where there was no other evidence of disease. The number of clear cells in the basal layer was markedly reduced in all these depigmented areas. The use of the S100 antigen labelling technique to identify melanocytes revealed an almost complete absence of labelled cells from the basal layer in the hypomelanotic macules.



FIGURE 1. Macular depigmentation over the upper trunk.

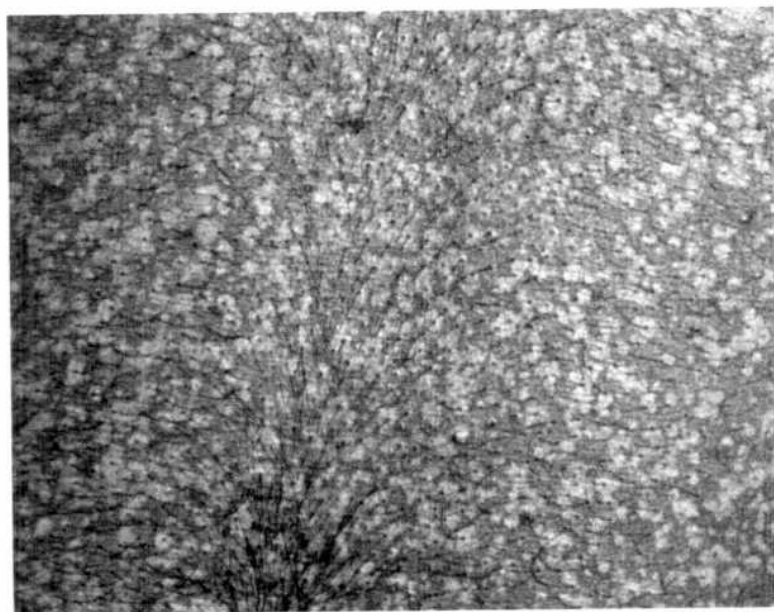


FIGURE 2. Depigmented macules on the lower chest closely related to hair follicles.



FIGURE 3. Warty lesions around the knee.

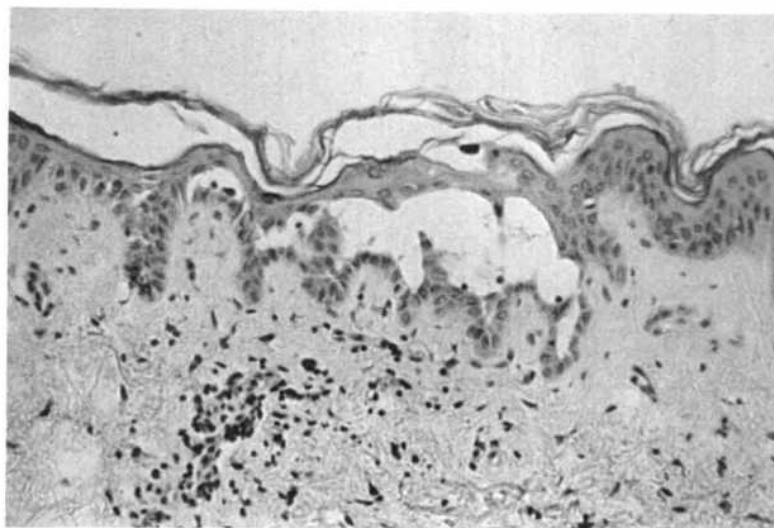


FIGURE 4. Histology from a depigmented macule. (H/E $\times 100$). Supra-basal cleft containing acantholytic cells.

DISCUSSION

The presence of macular depigmentation in Darier's disease has been the subject of only three previous reports,¹⁻³ and seems to be very rare. Goodall *et al.*¹ described macular depigmentation on the trunk and upper limbs of an American negress but did not comment on any relationship to the hair follicles. Cattano² reported a 54-year-old American negro with macular depigmentation occurring in close association with follicular and non-follicular hyperkeratotic lesions over the face, neck and trunk. Cornelison *et al.*³ reported three cases of Darier's disease

in American negroes with macular depigmentation affecting the trunk and extremities. In the latter report, no comment was made as to whether the distribution was peri-follicular, except that in one case, the lesions were both follicular and interfollicular.

From these reports certain consistent features emerge. In all cases the distribution seems to have been fairly widespread with particularly prominent involvement of the trunk. In all cases the depigmented lesions were macular and very small, being variously described as tiny, 3-4 or 1-5 mm in diameter. In our case both the distribution and size of the lesions are similar. A striking difference in our case is the marked peri-follicular pattern of depigmentation which has not previously been emphasized.

In previous reports, the histology of the hypomelanotic macules showed no features of Darier's disease.^{2,3} In both reports there was reduced pigmentation but no specific reference to the numbers of melanocytes. However the presence of atypical melanocytes in typical hypertrophic lesions was noted.² The case reported here is, therefore, the first in which the typical histological features of Darier's disease have been found in the hypomelanotic macules. Since no hyperkeratotic papules were present this represented sub-clinical disease. The evidence from conventional histology and the S100 stain indicate that in this case the hypopigmentation was due to an absence of melanocytes in the affected areas.

There is some controversy regarding the mechanism of the depigmentation. Cornelison *et al.*³ noted that the depigmented macules in their three patients were not at the sites of previous hyperkeratotic lesions. Since their histology revealed 'only reduced pigmentation and no evidence of Darier's disease they concluded that the depigmentation was not 'post-inflammatory' in nature. However, in the case reported by Cattano,² depigmented macules were present in close association with hyperkeratotic lesions. Adjacent hyperkeratotic and depigmented areas were biopsied and typical changes of Darier's disease were found in the hyperkeratotic area where there were abundant 'atypical' melanocytes. The depigmented area showed epidermal thinning as well as reduced pigmentation and he concluded that the macules were 'post-inflammatory' in nature, marking the site of previous hyperkeratotic lesions.

Reduced pigmentation is a recognized histological feature of Darier's disease⁴ and the finding of typical Darier's histology in some of the depigmented macules of our patient suggests that the loss of pigmentation is associated with past or present sub-clinical disease activity in this case, even though there is no history of typical hyperkeratotic lesions in the same distribution, and none have been seen during ten years of follow-up. It therefore seems possible that the leukoderma in Cornelison's patients was a sequel to previous sub-clinical disease, although there was no disease activity at the time of his biopsies. It may be that the atypical melanocytes noted by Cattano represented damaged cells and the apparent absence of melanocytes in the lesions of our patient reflects a later stage in the same process.

The peri-follicular distribution of the depigmentation in our patient presumably reflects the tendency for Darier's disease to affect follicular orifices and its synonym of 'keratosis follicularis'. Paradoxically, in the experience of the authors, the hyperkeratotic lesions of Darier's disease are usually not markedly peri-follicular.

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Post-irradiation morphoea

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SUMMARY

We present details of nine patients who developed morphoea after radiotherapy. In every patient morphoea began within the irradiated area and in four spread beyond it. We believe the irradiation triggered the morphoea despite the absence of any clear-cut relationship to dosage or severity of the acute reaction. Dermatologists and radiation oncologists should be aware that this condition may lead to the mistaken diagnosis of a local tumour recurrence.

X-rays were discovered by Roentgen in 1895 and were soon used to investigate and treat a variety of human diseases. The sequelae of such radiation, including atrophy and squamous cell carcinoma of the skin, had become obvious by the early part of this century. Radiation-induced morphoea was mentioned in the dermatology text books of that era (Barthelemy, quoted by Crocker, 1905),¹ but since then awareness of this condition has faded, being represented in current texts by a single, personally communicated case.² We wish to revive this awareness by presenting a series of nine cases. It is important to distinguish this condition from a sclerotic recurrence of the original tumour.

CASE REPORTS

Patient 1

Mrs S.E., aged 85 years, presented with right axillary lymphadenopathy. Histologically the glands contained adenocarcinoma. No primary tumour was found, however, and she underwent radiotherapy to the right axilla and supraclavicular fossa. Two years later a yellow triangle of firm skin appeared in the axilla with its base on the edge of the irradiated skin (Fig. 1). Biopsy showed the histological features of morphoea. The lesion extended medially, outside the radiation area. Its size has not changed and after 1 year it became softer. The maximum tissue absorbed dose of radiation in the area that developed morphoea was 45 Gray (Gy) in 20 fractions in 26 days.

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Patient 2

Mrs G.M., at the age of 55 years, developed carcinoma of the left breast with axillary node involvement. Mastectomy was followed by irradiation and eight cycles of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil. Three years later a shiny, yellowish-white area appeared, below the mastectomy scar, in the irradiated zone. Histology showed hyaline sclerosis in keeping with the clinical diagnosis of morphoea. She had previously developed rib fractures and constrictive pericarditis, both attributable to the radiotherapy and possibly aggravated by the chemotherapy she had received. The maximum tissue absorbed dose was 43.5 Gy in 10 fractions over 38 days.

Patient 3

Mrs J.F., aged 67 years, underwent wide excision of a right breast carcinoma, followed by radiotherapy to the right breast and peripheral lymphatics, followed by a high dose boost to the tumour bed in the lower, inner quadrant of the breast. Eighteen months later a bruise-like change was noted in the irradiated area in the upper central part of the breast; i.e. not in the high dose boost area. The changes, which persisted, had the clinical features of morphoea and the diagnosis was confirmed histologically. The maximum tissue absorbed dose in the area that developed morphoea was 45 Gy in 20 fractions over 29 days, and in the boost area was 60 Gy in 25 fractions.

Patient 4

Mrs M.T. developed left breast cancer in 1980 at the age of 44, and was treated by mastectomy and radiotherapy to the chest wall and peripheral lymphatics. In 1984 a recurrence was detected in the supraclavicular fossa and treated by tamoxifen. In 1987 she was noted to have white atrophic but indurated changes in the skin. The clinical and histological features were in keeping with morphoea. The maximum tissue absorbed dose was 45.7 Gy in 10 fractions over 28 days.

Patient 5

Miss P.B. was aged 59 when she underwent the local excision of an adenocarcinoma of the right

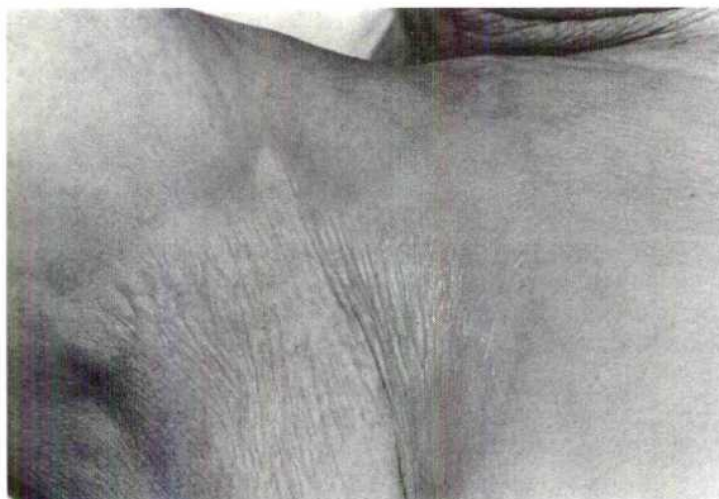


FIGURE 1. Patient showing post-irradiation morphoea.



FIGURE 2. Patient 7 showing post-irradiation morphoea.

breast, followed by radiotherapy to the breast and peripheral lymphatics. Some thickening of the scar was noted at 1 year and, after 2 years, nodules appeared in the upper part of the breast. Local recurrence was suspected but a biopsy was negative for tumour. These nodules increased over a further 2 years as a waxy plaque extending beyond the irradiation field. Morphoea was confirmed histologically and later accepted clinically at the 68th Annual Meeting of the British Association of Dermatologists.³ The maximum tissue absorbed dose to the breast was 59.4 Gy in 27 fractions over 43 days followed by a 10 Gy boost in a further 5 fractions to the tumour bed.

Patient 6

Mrs K.R., aged 67 years, underwent wide excision of a carcinoma from the upper half of the right breast followed by radiotherapy to the breast and peripheral lymphatics. A seroma was aspirated several times from the breast during therapy. Eighteen months later an erythematous rash was noted in the axilla. Twenty-seven months after treatment, a bruise-like change was noted around the scar extending to the midaxillary line and inferiorly beyond the irradiated skin. A biopsy confirmed the clinical diagnosis of morphoea. The maximum tissue absorbed dose was 50 Gy in 25 fractions in 42 days.

Patient 7

Mrs McD., aged 39 years underwent mastectomy and post-operative chest wall irradiation for a 1 cm breast cancer in 1974. In 1984 severe late radiation changes and areas of pallor were noted on the chest wall. Subcutaneous thickening, telangiectasia and a dappled pale yellowish appearance consistent with morphoea were found, but biopsy was contraindicated by the severity of the other radiation changes (Fig. 2). The maximum tissue absorbed dose was 45 Gy in 10 fractions over 28 days.

Patient 8

Mrs. B.D., aged 42, was treated for stage IB adenocarcinoma of the endocervix by low dose external beam radiotherapy to the pelvis followed by two radioactive caesium implants. Three years later she developed thickening of the skin over the left buttock and a separate area over the

left anterior superior iliac spine. A biopsy was consistent with the clinical diagnosis of morphoea and also showed some changes due to irradiation damage. The radiation dose to skin from intracavitary caesium insertions would have been minimal. The skin dose from the external beam treatment would have been less than 20 Gy in 16 days, and it is unlikely that the anterior superior iliac crest was in the beam.

Patient 9

Mrs. R.T., aged 65 years, was treated in 1986 for an invasive carcinoma of the left breast with radiotherapy and tamoxifen. She received a total maximum skin dose of 5.5 Gy to the left breast and axilla over 22 treatments. The left supraclavicular fossa was treated with cobalt, 5.94 Gy over 27 days, and a cobalt top up of 2 Gy to a 10-cm diameter field on the left breast was given with 10 treatments. Eighteen months later she noticed erythema of the left breast, which, over the following 4 months, developed into a plaque with the clinical features of localized morphoea.

DISCUSSION

The underlying cause of morphoea is still unknown but the notion of injury as a trigger is not new and the old synonym for morphoea—Addison's keloid—is a reminder of this. In one series of 191 patients with localized plaque or linear morphoea, 14 reported a previous injury (though not always at the site of disease) and in five the disease occurred near to an operation site.⁴ Further reports record other types of skin damage as an aetiological agent;⁵ in one individual it appeared after varicella⁶ and one man with cirrhosis developed morphoea at the site of Vitamin K injections.⁷ In one patient with established morphoea disease appeared at the site of penicillin injections.⁸

These reports are similar to those describing localizing factors in lichen sclerosus et atrophicus (LSA). Morphoea may coexist with LSA and both are associated with an increased prevalence of autoantibodies: indeed some people regard them as variations of the same disease. LSA has been found in the post-mastectomy field of radiation⁹ and exhibited the Koebner phenomenon in seven out of 395 cases.¹⁰ We feel that acceptance of this phenomenon should now be extended to include post-irradiation morphoea.

The mechanism of the association remains uncertain. The position has not changed much since Osler wrote of morphoea in 1898 that 'we really know nothing of the essential causes, and the data are not yet at hand upon which a satisfactory theory can be based'. However, a fibroblastic response to irradiation is well recognized. Radiation pneumonitis, for example, may progress to fibroblast proliferation in the lung. Radiation nephritis may lead to a progressive fibrosis ending in renal failure. Similar changes in the dermis may act as a stimulus to morphoea formation.

The age of our patients with radiation-induced morphoea ranged from 39–85 years. In every case the morphoea began in the radiation zone, but extended slowly beyond it in four cases. These changes do not appear to be related to total dose, as they developed at doses both below 20 Gy and above 69 Gy. In one patient, the morphoea did not develop in the higher dose boost area but elsewhere. Nor did its development relate to dose per fraction, which is an important factor in predicting other late effects such as subcutaneous fibrosis and osteitis: such changes are commoner when higher doses per fraction are delivered.^{11,12} In only three of our nine patients was the dose per fraction above 4 Gy. The tendency today is to avoid doses much above 2 Gy. Furthermore the morphoea was not related to the severity of the acute radiation reaction. In only two cases (Nos. 5 and 7) was the acute reaction of desquamation and erythema described as

severe, while cases 1, 3 and 8 experienced, at most, only mild erythema, and cases 2 and 4 developed moderate erythema with small areas of desquamation.

Five of our patients were seen, over a 14 year period, in the Radiation Oncology Unit in Edinburgh where more than 300 patients each year undergo chest wall or breast irradiation. The incidence of post-irradiation morphoea, therefore, is likely to be low but cannot be calculated precisely from our study. The condition is not debilitating, and we suspect that our figures may be an underestimate. Dermatologists and radiation oncologists should be aware of this condition that may lead to the mistaken diagnosis of a local tumour recurrence.

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Naevoid psoriasis

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SUMMARY

A six-year-old boy presented with an eruption comprising multiple psoriasiform plaques, arranged in linear bands distributed along the lines of Blaschko and confined to the left side of the body. We believe that the eruption is true psoriasis, occurring in an unusual naevoid distribution. The existence of a genuinely naevoid form of psoriasis has frequently been debated, and has tended to be discounted. We believe that we have encountered a child who has this condition.

CASE REPORT

The patient was first seen by us at the age of 2 years. His skin was normal at birth, but at the age of 3 months he developed cutaneous lesions on the left leg, followed by similar lesions over the next few months on the left side of the trunk and the left arm. This progressive extension of the eruption ceased by the age of 1 year. The lesions appeared to be slightly itchy. His parents noticed that the eruption temporarily improved during the summer particularly during a holiday in Greece. His general health had been excellent, and there was no family history of any skin disease.

On examination at the age of 2 years, there were numerous erythematous-squamous cutaneous plaques, most of which took the form of broad bands that roughly followed Blaschko's lines. The lesions were confined to the left side of the body, with a marked mid-line cut-off (Fig. 1). There was no scalp, nail or mucous membrane involvement. The remainder of the physical examination revealed no other abnormalities.

A skin biopsy was taken from a typical truncal lesion and this showed psoriasiform acanthosis with parakeratosis, occasional Munro micro-abscesses, and tortuous dilated capillaries in the dermal papillae (Fig. 2). These features were reported as fully consistent with psoriasis. His parents declined further skin biopsies for fibroblast culture to seek cytologically apparent chromosomal mosaicism.

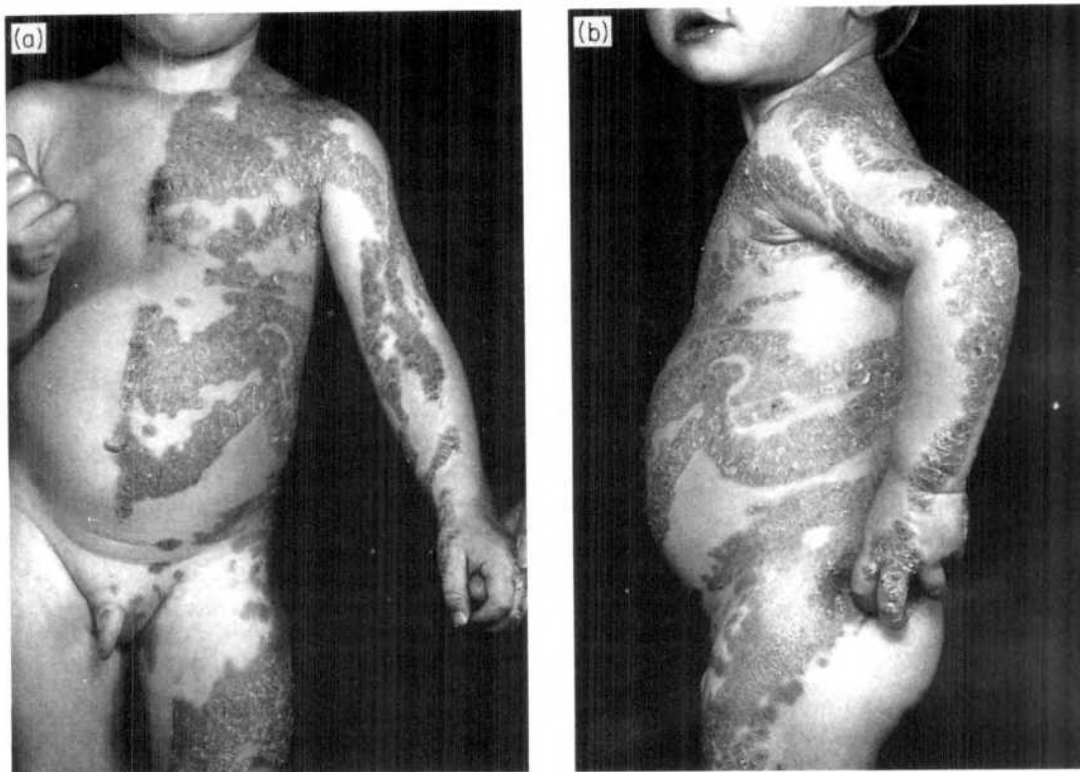


FIGURE 1(a) and (b). The appearance of the trunk and left arm at the age of 2 years, showing broad bands following Blaschko's lines, and having a sharp mid-line cut-off.

We considered that a diagnosis of psoriasis best fitted the clinical and histological findings, despite the distinctly naevoid distribution of the lesions. A course of phototherapy with short-wave ultraviolet light (UVB) was beneficial with individual lesions shrinking in size to leave hypopigmentation (Fig. 3). The eruption never disappeared and whenever phototherapy was discontinued it would again worsen. During this period, his parents noticed that the eruption became more severe immediately after an upper respiratory tract infection. Following his first exposure of the summer to natural sunlight, he developed a confluent erythematous rash in the exposed areas, that subsequently faded away over a period of a few weeks. A few months ago, we initiated short-contact topical therapy with dithranol in Lassar's paste, applied daily to selected areas for a period of 1 h, in concentrations which were gradually increased from 0.1% to 1.0%. This therapy proved very effective. We believe that there would have been no difficulty in clearing the eruption completely with this form of treatment. However, because the patient is himself not overly concerned by his skin disease, and also as it is now showing a distinct tendency to spontaneous improvement, we have decided not to attempt therapeutic clearance with dithranol.



FIGURE 2. Low power photomicrograph showing psoriasiform acanthosis with focal parakeratosis, suprapapillary thinning of the epidermis, and a small collection of pyknotic nuclei in the stratum corneum ($\times 150$).

DISCUSSION

We believe that this child has psoriasis, though the presentation is unusual. Apart from their distribution, the individual lesions are clinically and histologically indistinguishable from those of ordinary plaque psoriasis. The skin lesions have shown many of the behavioural characteristics of psoriasis, notably the isomorphic phenomenon following sunburn, and a good therapeutic response both to phototherapy and to dithranol. Alternative diagnoses include, (i) invasion of an epidermal naevus by psoriasis as a result of the isomorphic phenomenon, and (ii) an inflammatory epidermal naevus. Epidermal naevi that have been 'invaded' by psoriasis, as a manifestation of the isomorphic response, have been reported on several occasions.¹⁻⁴ It is argued that epidermal naevi are excellent psoriagenic foci, providing fertile sites for the development of psoriasis in suitably predisposed individuals.^{1,2,4} The psoriasis in these cases has generally made its initial appearance in a pre-existing verrucous epidermal naevus, with more typical psoriatic lesions appearing later at other sites. In these cases, the invasion of the



FIGURE 3. The left side of the trunk following a course of UVB phototherapy at the age of 5 years, showing great improvement of the lesions with residual hypopigmentation.

epidermal naevus by psoriasis initially occurred between the ages of 6 months and 63 years. In two cases, no residual histological features of the original epidermal naevus remained,^{1,3} but in the other two cases, biopsy showed typical features of psoriasis within a verrucous epidermal naevus.^{2,4} Therapy with intravenous methotrexate,¹ dithranol and ultraviolet light,² or topical corticosteroids,³ cleared the psoriatic element in these lesions, whereas the original epidermal naevus changed very little. Our patient developed psoriatic lesions without any sign of a preceding lesion. Furthermore, where lesions cleared either with phototherapy or by topical dithranol, the skin in the previously affected areas was perfectly normal, apart from post-inflammatory hypopigmentation.

A distinctive inflammatory type of epidermal naevus is now recognized, which is most often called 'inflammatory linear verrucous epidermal naevus' (ILVEN),⁵⁻⁸ though the more economical terms 'dermatitic', or 'eczematous' naevus are in some ways preferable.^{9,10} These lesions take the form of intensely pruritic linear groups of excoriated eczematous papules, which prove highly refractory to therapy. A rather characteristic histological picture of columns of hypergranulosis with orthokeratosis alternating with columns of agranulosis with parakeratosis has been described,⁶ though these features may not be observed in every biopsy. We do not feel that the lesions present in our case are of the ILVEN type because pruritus was minimal, the typical histological features were absent and the response to treatment was good.

We consider that our patient has psoriasis, which is confined to specific band-like zones of the skin. These zones appear to be distributed along the lines described by Blaschko.¹¹ Perhaps the most likely explanation for this phenomenon is that the child demonstrates mosaicism for the same genetic predisposition that is universally present in the skin of the ordinary psoriatic.

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Skin necrosis in giant cell (temporal) arteritis: report of three cases

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SUMMARY

Three elderly, female patients presented with scalp ulceration. One developed sudden blindness before the ulceration of the scalp. Biopsy from the ulcerated area in this patient did not show evidence of giant cell arteritis, but the two other patients had histological evidence of giant cell arteritis and one of these also became blind.

Giant cell arteritis is a disease of the elderly and is probably more common than previously thought. It is a disease of protean manifestations. Unexpected fever, malaise, depression, anaemia, carpal tunnel syndrome, headaches and vague musculo-skeletal pains and aches in the elderly should raise the suspicion of giant cell arteritis.

A high ESR is useful in making a diagnosis but it is not always elevated. Palpable tender arteries in the temporal region and scalp may be present but the pathological abnormalities are initially confined to short segments of the blood-vessels and frequently biopsies are inconclusive. Ulceration of the scalp, facial skin, along with ulceration of the tongue is uncommon and may cause diagnostic difficulties. We report three patients with ulceration of the scalp being a complication of giant cell arteritis.

CASE REPORTS

Case 1 (Mrs J.W.)

This 83-year-old woman first presented to an ophthalmic surgeon in May 1983 with sudden bilateral loss of vision over the preceding 48 h. Over the previous months she had had headaches and pain while chewing her food. On examination, there was no perception of light, the blood-vessels were narrow and there was evidence of lens opacities. The ESR was 104 mm/h. The histology of a biopsy of the right temporal artery did not show evidence of a giant cell arteritis. She was started on prednisolone, 40 mg daily and the ESR fell to 39 mm/h. She felt better but remained blind. Six months later she was seen by a psychiatrist with depression, auditory

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FIGURE 1. Large, irregular, ulcerated and crusted area on the whole vertex of the scalp.

hallucinations and paranoid delusions, which was thought to be due to her steroid therapy and the dose of prednisolone was reduced to 15 mg daily and later to 2.5 mg daily. Her ESR in March 1984 was 22 mm/h and at that time her prednisolone was discontinued. That month she developed ulceration on the vertex of the scalp which was thought initially to be self-inflicted (Fig. 1). The ESR at that time was 37 mm/h. Histology of a biopsy from the ulcerated area did not show evidence of giant cell arteritis but she was started on prednisolone, 15 mg daily and azathioprine, 100 mg daily. Over the next year her scalp ulceration healed and treatment was then discontinued.

Case 2 (Mrs W.E.)

This 71-year-old woman was admitted to a geriatric ward in September 1983 with a 2-month history of headaches on the right side of the head and on admission had a large crusted area on the right temporal region of the scalp, that measured 7 cm in diameter. It was tender and infected. Her vision was normal and her ESR was elevated, being 45 mm/h. A biopsy of the ulcerated area showed the classical histology of temporal arteritis. She was started on prednisolone, 60 mg daily and after 3 weeks this was gradually reduced to 10 mg daily. The ulcerated area healed and in October 1984 prednisolone therapy was discontinued. When seen again 6 months later the previously ulcerated area remained healed and the ESR was normal.

Case 3 (Mrs K.S.)

This 78-year-old woman was seen by a physician in February 1979 with a history of stiffness of

her arms, shoulders and fingers for 5 weeks that was associated with weight loss. She was admitted to hospital and was found to have mild cardiac failure, mitral incompetence and aortic stenosis. Her Hb was 11.3 g/dl, ESR 34 mm/h, white cell count 9,800. She was treated for her heart failure and improved and discharged home after a week. In March 1980 she suddenly went blind in the right eye and 2 days later lost the vision in the left eye. She had, at this time, developed a scaly, crusted and ulcerated lesion on the right parietal area of the scalp. On admission to hospital her ESR was 41 mm/h, Hb 11.3 g/dl. She was totally blind and there was a large, crusted, ulcerated area on the right parietal region of the scalp, measuring 7 × 5 cm in size. The histology of a biopsy from the ulcerated area in the scalp showed a giant cell arteritis. She was started on prednisolone, 40 mg daily and by October 1980 her scalp lesion had almost healed. She remained on prednisolone for 1 year, her ESR remaining normal. In September, 1980, she developed carcinoma of the vulva that was treated with radical vulvectomy but in October 1987 she was admitted to a geriatric ward with an extensive local recurrence and she died a few weeks later.

DISCUSSION

Horten *et al.*¹ were the first to define temporal arteritis although Hutchinson² is credited with describing the first case. Giant cell arteritis is a systemic granulomatous vasculitis, usually occurring in patients between the ages of 65 and 73 and more common in women.⁴⁻⁸ It may involve large, medium or small sized arteries as well as veins,⁸⁻¹⁰ the most frequently involved vessels being the large and medium sized arteries from the aortic arch to the scalp.⁷ The typical histological picture is also seen in the arteries of the uterus,¹¹ legs,¹² abdomen³ and hands.¹³ The most significant laboratory finding in giant cell arteritis is a moderate to marked increase in the ESR, sometimes exceeding 100 mm/h,⁸ but a normal ESR has been reported even with acute disease.¹⁴ The prevalence of temporal arteritis with a normal ESR is not known but has been estimated at being from 2 to 8.7%.¹⁴

Necrosis of the skin of the scalp in giant cell arteritis is rare. It may be localized to one side, or may be bilateral¹⁵⁻¹⁷ and it may extend to a large area with much of the scalp being involved^{18,19} as occurred in our first case. Sixty-seven percent of patients with scalp necrosis will also develop visual loss and the mortality rate is higher than in those without necrosis.¹⁹ Siemssen²⁰ reports on 49 cases of necrotic lesions in the scalp with ulceration of the facial skin and tongue and in 29 there was histological proof of giant cell arteritis. In all our patients there was extensive ulceration of the scalp and in two, the diagnosis of giant cell arteritis was confirmed by biopsy. Temporal arteritis with scalp ulceration and a normal ESR and without histological evidence of giant cell arteritis has recently been reported.²¹ Patients who are suspected of the diagnosis of giant cell arteritis should be started immediately on steroid therapy and a temporal artery biopsy can be later obtained.²² Delay in treatment may be disastrous and result in the patient going blind. Scalp necrosis is a rare presentation of temporal arteritis which often has a worse prognosis.¹⁹ Though other conditions may produce ulceration of the scalp, one should have a high degree of suspicion for temporal arteritis, particularly in the elderly, and appropriate treatment should be given.

ACKNOWLEDGMENT

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Obituary

Dr Louis Forman MD, FRCP

Louis Forman was born on the 15th June 1901 at Portsmouth. Louis or 'Lolly' as he was often known, entered Guy's as a medical student in 1918 and soon after, found himself wearing the arm band of a special constable carrying a truncheon and policing the Armistice Day celebrations in Hyde Park. He was a brilliant student winning prizes and distinctions and was to be associated with Guy's Hospital and Medical School for 70 years. Qualifying in 1923, he became houseman to Sir Alfred Fripp and Dr Fawcett, and later worked with Sir Herbert French and Dr (later Sir) Arthur Hurst. From 1926 to 1930 he was a general physician at St Olave's Hospital. Geoffrey Dowling was the visiting dermatologist and a great influence taking him to his other hospitals. In 1930 he returned to Guy's as registrar to the Department of Dermatology, where he fell under the spell of H.W.Barber. In 1932 Dowling left Guy's for St Thomas' Hospital and Louis became Assistant Physician to the Department. In 1935 he also became a member of



Dr Louis Forman MD, FRCP.

the staff of St John's Hospital for Diseases of the Skin. In 1939 war broke out and Guy's Medical School moved to Farnborough in Kent and Louis became the Sub-Dean and also a little later, an Acting-Major in the Home Guard. Here he taught medicine as well as dermatology and would lead groups of students around the hospital, ending up in the isolation huts at the bottom of the garden where a patient with exfoliative dermatitis or another with Hansen's disease might perhaps be found. He was consultant dermatologist to the South East Sector of the Emergency Medical Service and was responsible for a dozen hospitals and more than 100 service patients at a time. After the war he gave up his sector work and in 1946 when Barber retired he became Senior Physician to the Department. In 1948 the National Health Service was started and Louis helped to set up services at his old Sector hospitals at Dartford, Orpington, Farnborough and Sevenoaks. Young doctors came back from the war and a number were attached to the Department for training. They were lucky to have Louis as a mentor. Patient, gentle, wise and tolerant, he was keen that they should have the valuable experience of research and he encouraged them to work in other departments in the Medical School. There are memories of 'The Director' as he was called at this time sitting in a sunlit room on the second floor of outpatients teaching students. He seemed to have all the time in the world to discuss or explain or indulge in his favourite occupation of thinking aloud. Any views or opinions, however immature, were gravely received and considered. Time passed and lunch or other meals seemed of little account. On Wednesday afternoons he and the registrars would sit in another room discussing histological slides, Louis being one of the pioneers in the use of biopsy and histology in this country. Selected patients were brought and those with specially obdurate oral lesions seemed to predominate, and on these Louis would direct a steady and extraordinarily prolonged gaze, so much so that his disciples came to believe that this was an essential tool of management. He had a level headed rational sort of mind and this combined with unusual powers of observation and an extraordinary clinical memory made an indelible impression. At the same time he had endearing foibles such as his exaggerated conception of the pathogenic powers of the *Demodex folliculorum* and the unshakeable belief that men with rosacea were invariably and severely hen-pecked. The excellent memory was tempered by occasional vagueness and forgetfulness, as when Louis appeared at Outpatients on a Bank Holiday and found it locked. The next day all and sundry were reproached for keeping the matter secret. He made contributions to medical knowledge beginning in 1927 with a report on 'The late results of Chronic Gastric Ulcer Treated Medically', and his classic paper on the aetiology of erythema nodosum appeared in 1946. Louis was much intrigued by the powerful biological effects of Thorium X, which seemed at odds with the low penetrance of the radiation. In 1952 he reported that Thorium X penetrated the intact skin and could be demonstrated in the blood and in various layers of the skin. This caused surprise because at that time the horny layer was regarded as a formidable and impenetrable barrier. In 1961 he gave the Watson Smith Oration at the Royal College of Physicians on 'The Action of Metals on the Skin' and in 1965 he delivered the Prosser White Oration on the subject of 'The Skin and the Colon', an astute analysis of a complicated subject. His other numerous publications were clinical and therapeutic and usually included elements of speculation in which he delighted. He was very conscious of his great predecessors at Guy's and when Joseph Towne's famous wax models returned to the Gordon Museum after underground storage in the war he catalogued them as well as the memorabilia of Addison, Bright, Hodgkin and Astley Cooper.

In 1960 he was President of the British Association of Dermatologists and enjoyed acting as host to the Franco-British Reunion of that year. In 1966 as President of the Dowling Club he led an expedition to Sweden which was a great success. He made many other excursions abroad and was Honorary Visiting Dermatologist to John Hopkins Hospital. He had been President of the St John's Hospital Dermatological Society and President of the Section of Dermatology of the Royal Society of Medicine. The latter recognized his merit by awarding him the distinction of Honorary Fellowship. He was an Honorary member of British Association of Dermatologists, the French, Israel, German and Swedish Dermatological Societies, and of the Society for Investigative Dermatology.

Lolly was a good and keen tennis player and a devotee of Wimbledon too. He was fond of music and as a talented pianist could make his own. He persuaded Ian Magnus to join him in a duet, Debussy's 'Queen of Sheba' on one occasion. (He had once expertly resuscitated Ian from an adverse shock reaction to an experimental carbachol injection.) He had a love affair with the motor car which was not always

reciprocated. He was interested in growing flowers when he lived at Chislehurst and he enjoyed sailing, the theatre and watching wrestling.

He retired from Guy's in 1967 and the following year colleagues and old pupils from Guy's published a Festschrift in his honour with contributions from his American friends Herman Beerman and Marion B. Sulzberger. After retirement he continued in private practice until failing vision made him give up. He continued until the end attending the meetings of the Section of Dermatology, and those of the St John's Hospital Dermatological Society and he had a special seat at the Saturday morning registrars' meetings at St John's. His memory and detailed case records enabled him to make a unique contribution on these occasions, reinforced by his care and concern for those in training.

In his last years a devoted friend read dermatology to him for several hours a day. He died on the 13th November 1988 in the hospital which he had served so long and well.

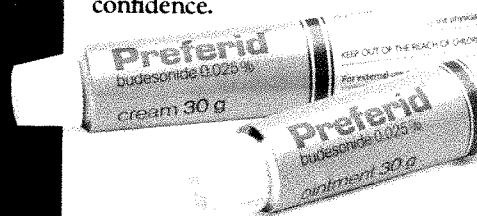
J.S.P.

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1. Andersson R, et al. J. steroid. Biochem. 1982; 16:787-95. 2. Edsbacker S, et al. Drug Metabolism and Disposition, Vol. 11, 6:590-6. 3. Scott M, et al. Acta Dermatovenereol (Stockholm). 1981; 61:543-6. **Prescribing Information:** Presentation: Preferid ointment contains budesonide 0.025% w/w. **Uses:** In the topical management of corticosteroid sensitive dermatoses such as eczema and psoriasis. **Dosage and Administration:** Apply a small quantity to the affected part two or three times daily. **Contraindications, warnings, etc:** Contra-indicated in the presence of bacterial (eg impetigo), viral (eg herpes simplex) and fungal (eg candida or dermatophyte) infections. Also in facial rosacea, acne vulgaris, perioral dermatitis, perianthelidosis and napkin eruptions. **Side-effects:** Local and systemic toxicity with topical corticosteroids is common, especially following long continued use on large areas of damaged skin, in flexures and with polythene occlusion. If used in childhood, or courses should be limited to 5 days and occlusions should not be used. **Use in pregnancy and lactation:** There is inadequate evidence of safety in human pregnancy. Topical administration of corticosteroids to pregnant animals can cause abnormalities of development including cleft palate and intra-uterine growth retardation. There may therefore be a very small risk of such effects in the human foetus. **Other warnings and precautions:** Long term continuous therapy should be avoided in all patients following development of tolerance, risk of generalised pustular psoriasis and local and systemic toxicity due to impaired barrier function of the skin. Steroids may have a place in psoriasis of the scalp and chronic plaque psoriasis of the hands and feet. Careful patient supervision is important. **Overdosage:** Overdosage would result in the topical and systemic signs and symptoms associated with high corticosteroid dosage. If overdosage of this topical corticosteroid product would be unlikely to occur other than as a result of severe and prolonged abuse of the product. **Product Licence Numbers:** Preferid Cream 0166/0123, Preferid Ointment 0166/0122. **Product Authorisation Numbers:** Preferid Cream 62/27/1, Preferid Ointment 62/27/2. **Holder of the Product Licence/Authorisation:** Brocades/Great Britain/Limited, Brocades House, Pyrford Road, West Byfleet, Surrey KT14 6RA. **Basic NHS/GMS Prices:** Cream 30g £7.96, 100g £15.80, Ointment 30g £7.96, 100g £15.80.

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Dust- and rod-like particles in cutaneous biopsies of patients with systemic lupus erythematosus

SIR, We read with interest the article by Nieboer *et al.*¹ about their observation of dust-like particles (DLP) in skin biopsies of patients with subacute cutaneous lupus erythematosus (SCLE). We have also recently observed fine granular, dust-like, and in places rod-like particles (RLP) in biopsies of lesional skin of three patients with systemic lupus erythematosus (SLE). Relevant data about these patients are listed in Table 1. However, in contrast to the description of Nieboer *et al.* the detection of DLP and RLP in the biopsies of our patients was at the basement membrane zone (Figs 1a, 2a) and in and around dermal vessel walls (Fig. 1b) and most strikingly also in dermal tissue between the vessels (Fig. 1a, 2b). These structures can be overlooked and this occurred at the first reading of our specimens when only the lupus band and vascular immunoglobulin and complement deposits were observed. On histopathology there were in all three biopsy specimens dermal infiltrates of lymphocytes.

The following argue against the possibility that the DLP and RLP represent artefacts as previously discussed.¹ An identical immunofluorescence staining pattern was found when cryostat sections of the same skin biopsy were processed in two different laboratories (Institute for General and Experimental Pathology and Department of Dermatology, University of Innsbruck, Austria) and read independently by different investigators. Immunofluorescence studies of the same biopsy were performed on different days with the same result and DLP and RLP were not observed in skin biopsies of patients with various other dermatoses, nor in two other patients with SLE processed in parallel with or using the same antibody dilutions. Furthermore repeated biopsies of the same patient yielded identical results and with non-relevant FITC-conjugated antibodies (OKT6, anti-HLA-DR) the expected staining pattern of epidermal and dermal cells but not of DLP and RLP were observed.

We believe, like Nieboer *et al.*,¹ that these DLP and RLP represent immune complexes deposited in the intervascular dermis in patients with a large quantity of circulating immune complexes (CIC) in their serum. This is supported by the fact that all three patients had CIC detectable in their sera. The role of

TABLE 1.

	Patient 1, male, 62 years	Patient 2, female, 28 years	Patient 3, female, 32 years
Antinuclear antibodies (ANA) on Hep 2 cell substrate	IgG-ANA: 1:640 IgM-ANA: 1:10	IgG-ANA: 1:10240 IgM-ANA: 1:80 IgA-ANA: 1:20	IgG-ANA: 1:10240 IgM-ANA: 1:10
ANA-subsets (SS-A, SS-B, RNP)	Sm, RNP: negative SS-A: positive SS-B: negative	Sm, RNP: negative SS-A, SS-B: negative	Sm, RNP: positive SS-A, SS-B: negative
Anti-native DNA	Negative	1:2560	1:2560
Circulating immune complexes (CLq binding assay, normal 5 µg/ml)	76 µg/ml	26.5 µg/ml	135 µg/ml
Direct immunofluorescence	Lupus band IgM, C3 Vascular deposits IgM, IgA, C3	Lupus band IgG, IgM Vascular deposits C3	Lupus band IgM Vascular deposits not detectable
Dust-like and rod-like particles	IgG, IgM, IgA, C3	IgM, C3	IgA, C3

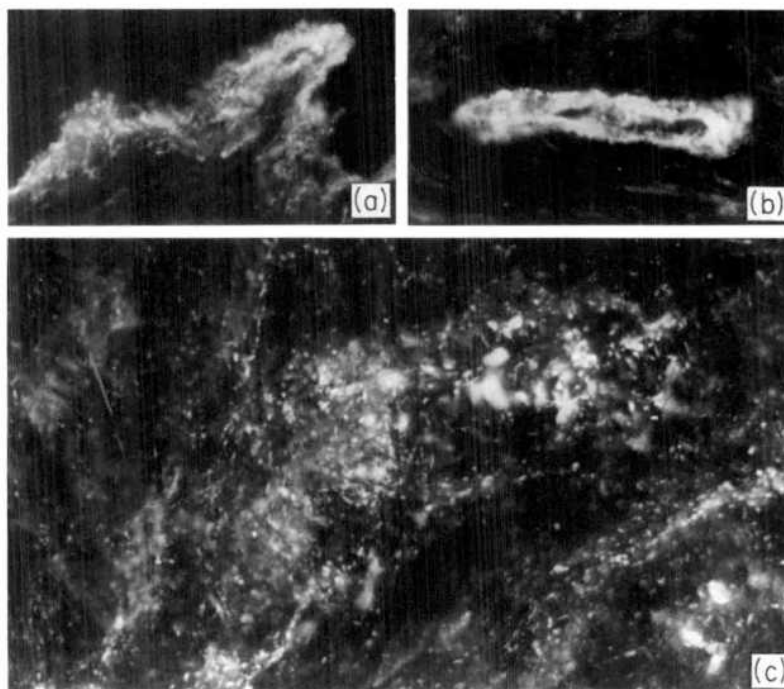


FIGURE 1. Patient 1: (a) IgM lupus band. (b) Vascular deposits of IgM. (c) Dust- and rod-like particles (IgM) in the lower dermis. (a, b and c $\times 250$).

antinuclear antibodies (ANA), also present in high titres (Table 1) in the patient's sera, in the formation of these CIC is unclear. However in the biopsy of patient 1, the staining of DLP and RLP disappeared after incubation with 0.1 M glycine at pH 3.0 for 1 and 4 h at room temperature and with 0.1 M sodium periodate, for 4 h at room temperature and indicated the elution of these deposited immune complexes. Transmission electron microscopy also revealed granular material in the dermis, which in places was located around elastic fibres and probably represented the ultra-structural equivalent of the particles observed on immunofluorescence. The presence of bacteria, viruses and fungi in the dermis was ruled out by electron microscopy. The localization of CIC in the dermis of mice was observed² in an interstitial pattern after injection of an intravenous bolus of large latticed immune complexes.

We believe that the presence of DLP and RLP on immunofluorescence skin biopsies of patients with CIC is frequently overlooked even by experienced personnel and that it is not specific for SCLE as has been suggested.

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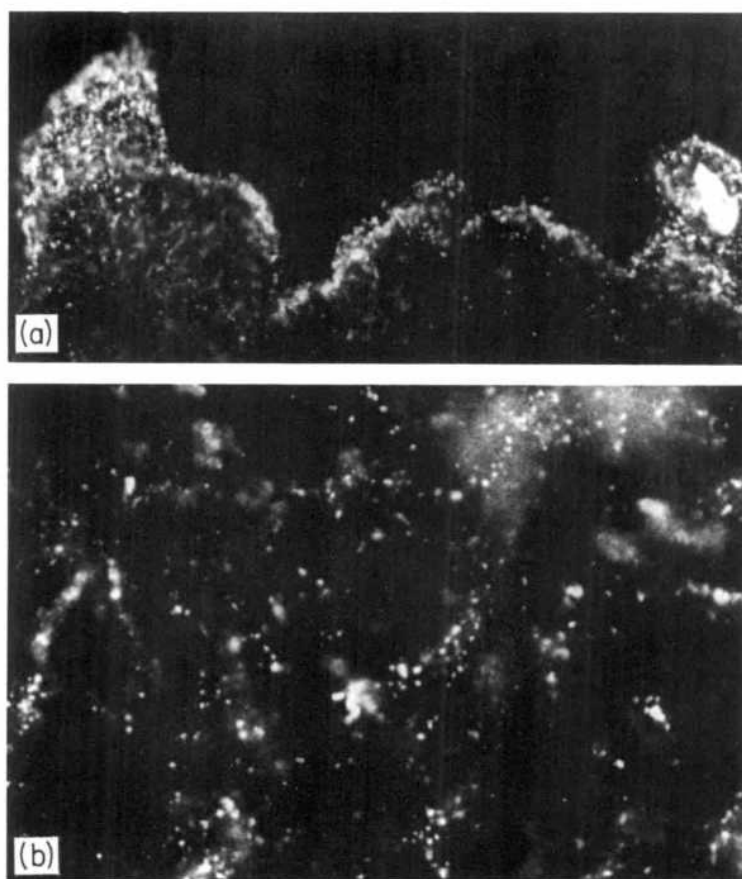


FIGURE 2. Patient 2: (a) IgM lupus band. (b) Dust-like particles (IgM) in the dermis. (a $\times 250$, b $\times 500$).

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Attempted passive transfer of a serum factor in chronic urticaria from human to monkey

SIR, The wheal and flare response to autologous intradermal serum in some patients with chronic urticaria provides evidence of a circulating pro-inflammatory mediator.¹ Passive transfer of the wheal and flare response from human to human has been demonstrated in one volunteer (unpublished) but for ethical reasons it has not been possible to repeat this observation. Because there have been two previous reports of successful passive transfer of a serum factor from human to monkey in symptomatic dermatographism² and in cold urticaria³ we attempted passive transfer of the serum factor in chronic urticaria into two species of Macaque monkey.

Sera from 10 patients with chronic urticaria showing a strong wheal and flare response to autologous serum, five patients with chronic urticaria showing little or no response, two patients with chronic urticaria in clinical remission and five control subjects without urticaria (two with psoriasis and three showing no evident skin disease) were studied. Sera were stored between 1 week and 29 months (mean 28.9 weeks $\pm$ 37.5) at -20°C and remained frozen until use. The panel of sera was tested on clipped thoracolumbar skin of three different monkeys, which were anaesthetized with intramuscular ketamine hydrochloride. (Fig. 1)

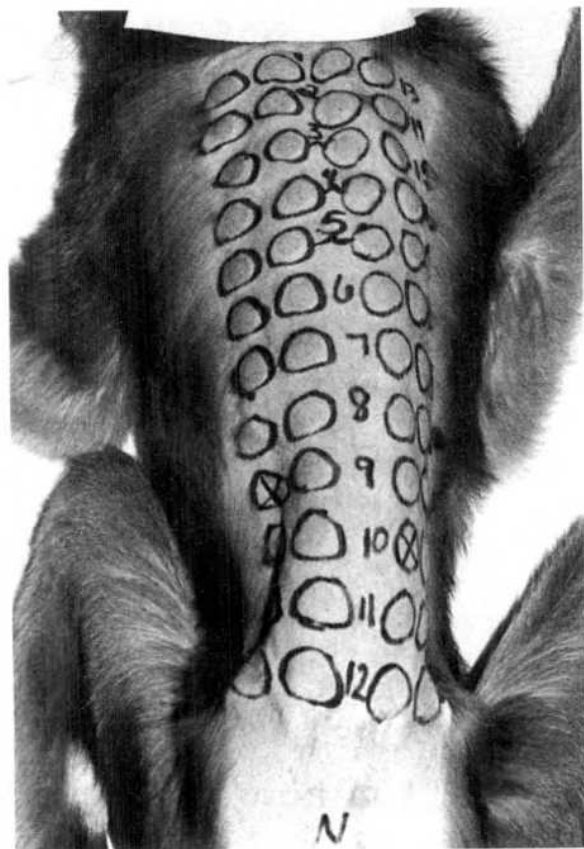


FIGURE 1. Macaque monkey in experiment attempting passive transfer of serum factor in chronic urticaria from human to monkey.

In the first experiment 0.2 ml paired aliquots of the randomized and coded sera were injected intradermally into a healthy 5 kg male *Macaca nemestrina* monkey 45 min after intravenous injection of 6 ml 2% Evan's Blue. Control skin tests of 0.2 ml histamine acid phosphate (2 μg) in normal saline and sterile normal saline were performed 15 min after injection of Evan's Blue to assess the blueing response. An identical protocol was observed for a 5 kg male *Macaca fascicularis* but here the skin tests were 0.05 ml. In the final experiment skin tests were repeated on a 5.6 kg female *Macaca nemestrina* using 0.1 ml aliquots 30 min before intravenous injection of 6 ml 1% Evan's Blue. Fresh serum from one control subject, histamine and saline were also skin tested after injection of the Evan's Blue. Measurements of the longest and shortest diameters of the blueing response were made at 30, 60, and 120 min and surface areas calculated. The skin test sites were also inspected 24 h after skin testing for late reactions.

Strong blueing responses were seen in both *M. nemestrina* and *M. fascicularis* at all serum test sites after

2% Evan's Blue injection, appearing within 10 to 15 min and changing little in size over 2 h. Although mean blueing areas were slightly greater with patients' sera than control sera at all time intervals with *M. nemestrina* the differences were not statistically significant (Table 1). No differences were seen with *M.*

TABLE 1. Mean surface area (mm²) of blueing response in paired skin tests for *M. nemestrina*

	30 min	60 min	120 min
Patients			
Serum-positive (10)	31.11	30.72	31.74
Serum-negative (5)	34.42	34.10	34.88
Remission (2)	34.18	33.78	34.96
All patients (17)	32.43*	32.07*	33.05*
Controls			
Sera (5)	29.85	29.70	30.48
Saline (1)	22.00	18.86	18.86
Histamine (1)	39.29	44.00	42.43

**P* = N.S. (Student's *t*-test) when compared to control sera at the same time intervals.

fascicularis. Blueing responses were seen with histamine and also occurred weakly with saline. When skin tests were performed 30 min before injection of 1% Evan's Blue palpable wheals were apparent within 10 to 15 min but no blueing responses developed in any of the serum, histamine or saline-injected sites. However, it should be noted that fresh serum from one control subject, histamine and saline injected intradermally in the same monkey after injection of 1% Evan's Blue produced similar blueing to that seen previously.

Macaca nemestrina and *fascicularis* were used to demonstrate passive transfer of dermatographism² whereas Misch *et al.*³ used a cynomolgous monkey, baboons and rhesus monkeys. In these studies Evan's Blue was injected up to 24 h after skin testing, but before challenge with the physical stimulus and this may explain why they did not report the non-specific blueing of the serum test sites seen in the present study. In those studies weights of the monkeys used in their experiments were not recorded so direct comparison of the dose/kg of dye is not possible. Although we were not able to demonstrate pro-inflammatory activity in the chronic urticaria sera with the protocol used in this study, passive transfer of reaginic antibodies cannot be excluded because subsequent challenge with antigenic or physical stimuli was not performed.

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ACKNOWLEDGMENT

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Calmodulin and psoriasis

SIR, The recent publication of Al-Ani *et al.* was another in the series of investigations about calmodulin (CaM) and psoriasis by the Sheffield group.¹

We would like to comment on two points: (a) the relationship to psoriasis *in vivo*, (b) the usefulness of CaM inhibitors in antipsoriatic therapy.

(a) It was described by Staiano-Coico *et al.*² that psoriatic plaques express a percentage of cycling cells (G2 + S + M) twice that of normal epidermis. This is accompanied by a doubling of the level of epidermal CaM.^{3, 4} Since CaM is involved in the progress of cells through G1,⁵ a linkage of both hyperproliferation and elevated CaM is likely. Experiments with partially hepatectomized rats showed an increased *de novo* synthesis of CaM in the pre-replicative phase of liver regeneration.⁵ As far as we know, comparable studies in psoriasis are lacking. We have performed an investigation on CaM in the epidermis of spreading lesions of psoriasis in order to determine the relationship of increase in CaM with the formation of the plaque.⁶ The highest CaM values were detected in apparently normal perilesional skin and were about three times higher than in the lesion itself. This suggests that the epidermal CaM increase precedes both the formation of a psoriatic lesion and the hyperproliferation.

It is conceivable that different cell lines, e.g. keratinocytes, fibroblasts, and B16 melanoma cells as used by Al-Ani *et al.*, might have a different kinetic of the pre-replicative CaM wave. In rat liver the maximum level of CaM is reached within 8–12 h, in psoriatic plaques it seems to be a permanent feature.^{3–5} This view might explain why Al-Ani *et al.* were unable to measure a CaM increase 3 days after plating fibroblasts and B16 melanoma cells in a high Ca^{++} medium.

(b) Al-Ani *et al.* performed *in vitro* studies on the effect of CaM inhibitors, i.e. W7 and derivatives on the proliferative activity and CaM content of keratinocytes. The authors measured the effects on CaM with a phosphodiesterase assay. W7 is a CaM inhibitor of group II according to Zimmer and Hofmann.⁷ That means that the K_i value for the activation of phosphodiesterase is about 10 times lower than the K_i for myosin light chain kinase. Furthermore, the activation of phosphodiesterase is readily blocked by occupation of a binding site in the alpha-helix of CaM. In contrast, the inhibition of other enzymes, possibly involved in the pathogenesis of psoriasis, requires binding of CaM inhibitors to a second site.⁷ Thus, inactivation of a CaM-dependent rise in phosphodiesterase activity *in vitro* by W7 does not imply any antipsoriatic effect *in vivo*, at least since other enzymes might be un-inhibited.

Though there is anthralin inhibition of the CaM-dependent phosphodiesterase *in vitro*,⁸ the same compound failed to inhibit the activation of the Ca^{++} -ATPase *in vitro* (U.Wollina, R.Klinger, R. Wetzker & B. Knopf, unpublished).

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Cyclosporin in the treatment of palmo-plantar pustulosis

SIR, Palmo-plantar pustulosis (PPP) is a chronic condition in which there are erythematous scaly plaques with sterile pustules affecting the palms of the hand and soles of the feet.¹ PPP is regarded by many authors as a form of psoriasis, although these two diseases do not share a similar HLA-antigen association.² PPP is resistant to most treatments, the best results being obtained with oral retinoids and PUVA.³ Encouraged by the good results of low-dose cyclosporin in psoriasis,⁴ we have treated six PPP patients (three male, three female, age 28-45 years) with low-dose cyclosporin at 2.5 mg/kg per day over a period of 1 to 3 months. An improvement or complete clearing of PPP occurred in all six patients for all the parameters studied with reduction of erythema, scaling and the formation of pustules. During treatment we saw occasional new pustule formation in all six of the patients but this slight relapse typically persisted for only 1 or 2 days. The disease activity was clearly diminished and we have not observed this with other treatments. No adverse effects were observed, such as hypertension or an increase in the serum creatinine. On stopping treatment with cyclosporin there was always a relapse within a few days. From this preliminary study we consider that low-dose cyclosporin at 2.5 mg/kg per day is an effective treatment for PPP and suggest that a larger controlled study with low-dose cyclosporin is needed.

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Fixed drug reactions to pseudoephedrine hydrochloride

SIR, The recent report by Taylor and Duffill (*British Journal of Dermatology* 1988; 118: 827-9) concerning a scarlet fever-like eruption caused by allergy to pseudoephedrine hydrochloride failed to mention that five patients have been reported in three separate publications who developed fixed drug reactions to pseudoephedrine hydrochloride.¹⁻³ In three cases, the nature of the eruption was confirmed with results of a challenge test.

Sympathomimetic drugs must be rare causes of drug eruptions in general, considering their frequent use in over-the-counter cold medications. However, it would appear that fixed drug eruptions, particularly the non-pigmenting variety, may be the most common cutaneous reaction to pseudoephedrine hydrochloride.

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Book Reviews

Vascular Birthmarks: Hemangiomas and Malformations. J.B. Mulliken and A.E. Young (1988) Philadelphia, London, Toronto, Montreal, Sydney and Tokyo: W.B. Saunders Company. Pp. 483; 363, illustrations. ISBN 0-7216-6601-9. Price £98.00.

This beautifully produced text book edited by a Harvard Professor of Surgery and a Consultant Surgeon from St Thomas's Hospital is aimed at the primary practitioner, family physician or surgeon who will usually have had little or no experience with the diagnosis and management of vascular birthmarks. It is also directed at the familiar and fascinated who will undoubtedly include all dermatologists.

The text consists of four parts and a comprehensive glossary and index which has many up-to-date references.

Part one discusses vascular birthmarks in folklore, history, art and literature and goes on to differentiate haemangiomas from malformations. This differentiation is crucial to the understanding of the text and was originally described by Professor Mulliken in 1982.

Part two concerns the diagnosis, natural history, pathogenesis and treatment of haemangiomas.

Part three discusses the pathogenesis of vascular malformations, their clinical and radiological assessment. There follows chapters on capillary (port wine), venous, arterial, lymphatic and combined vascular malformations.

Part four discusses problems and solutions concerning vascular malformations in the central nervous system, head and neck, upper and lower limbs and intra-abdominal vascular lesions. There are two good chapters on treatment with argon and tunable dye lasers and embolization of vascular malformations.

The book ends with a chapter on the emotional aspects of birthmarks by a psychiatrist.

This book is a pleasure to read and contains a wealth of information. The illustrations both in colour and black and white are uniformly excellent. They include photographs of Mikhail Gorbachev and a Picasso painting depicting a lymphatic malformation. This is a book to browse over and enjoy and would be a welcome addition to any dermatological library. It is strongly recommended to those who have an interest in birthmarks.

N. MORLEY

Year Book of Dermatology 1988. (1988) Chicago: Year Book Medical Publishers inc. Pp. 466. Price £37.50.

Every year at about this time I open my beak wide and let the editors of the Year Book of Dermatology fill it with predigested morsels from the literature, nicely seasoned by the comments of their team of experts.

Spoon feeding it may be, but it seems to suit me and the recipe has worked well for many years. Our departmental collection goes back to 1935, and is often consulted when a key reference from the past is needed. The Year Book is not a substitute for a regular diet of standard journals, but an extra. Ask yourself how many of the 700 journals surveyed by the editors you would normally read.

How does the current volume compare with those of other years? It is as good as ever. In 1983 I was pleased to see the back of the over-cooked review articles which used to make such a heavy first course: this year we have a three page review of lasers and some quirky tables of 'statistics of interest to dermatologists'. One of these tells us that the BJD has an unusually high 'impact factor'. I hope its book reviews have too; if so, take my advice and joint the annual feast.

J.A. SAVIN

Essential Paediatric Dermatology. J. VERBOV (1988) Bristol: Clinical Press. Pp. 182. Price £25.00.

In recent years there have been a number of concise textbooks in paediatric dermatology, all of a high standard and all profusely illustrated with colour photographs. How does one choose which of these is the best? There is no easy answer, but it is my opinion that for trainees in dermatology and paediatricians this one, *Essential Paediatric Dermatology*, is excellent. The text is succinct and the essentials are given on

diagnosis and treatment of common skin disorders as seen in the new born and the young child. Though a number of uncommon conditions are also described and indeed some of them are very rare, the emphasis is on those disorders that are frequently seen in the paediatric dermatology clinic. The chapter on atopic and other dermatitis is particularly useful and there is a balanced view on the management and treatment of this very common disorder. Though most of us believe that breast feeding does decrease the risk of eczema we see, not infrequently, the condition develop in exclusively breast fed infants. This topic is discussed without bias. A minor point is that mention should have been made on the various groups such as the National Eczema Society, Dystrophic Epidermolysis Bullosa Research Association (DEBRA) and the Psoriasis Association and others that offer advice and help to parents. Perhaps in the next edition these can be in an appendix. The colour photographs are mostly good with reasonable reproduction of the colour. A few give a rather long distance view of the lesions and could be replaced with better close ups.

Finally, the book is reasonably priced and a good buy for the dermatologist and paediatrician.

S.S.BLEEHN

Advances in Immunohistochemistry. Edited by R.A.DeLellis (1988) New York: Raven Press. Pp. 496. ISBN 0-88167-394-3. Price \$90.00.

This book is aimed primarily at pathologists, which is not to deter those dermatologists with an interest in immunohistochemistry from reading it.

There is comprehensive cover of the newer methods in this field including alkaline phosphatase, glucose oxidase, *in situ* hybridization, lectins and immunoelectron microscopy. These chapters have helpful appendices with detailed protocols which should enable the techniques to be used, although beginners in '*in situ*' would be ill advised to try to reproduce the complicated combined *in situ*/immunohistochemistry methods solely from this book.

Chapters covering the application of immunohistochemistry to diagnosis are well written, with useful tables of antibody reactivities and interpretation of results based mostly on the well known immunoperoxidase method. Those on monoclonal antibodies and human carcinoma, the diagnostic application of keratins and leucocyte common antigen are particularly good.

Unfortunately for a book on techniques such as this, the photographic illustrations in some of the chapters are disappointing. This detracts somewhat from an otherwise well written and informative reference book for diagnostic pathologists.

MARLYN L. TURBITT

Adverse Drug Reactions in Dentistry. R.A.SEYMOUR AND J.G.WALTON. Oxford: Oxford University Press. Price £18.95. ISBN 019 2616 17.

This book is a most welcome addition to the literature and has condensed current knowledge of oro-facial drug reactions in a concise and readable manner. The book is divided into five main chapters covering unwanted effects of drugs used in dentistry, adverse drug reactions affecting the mouth and associated structures, prescribing to the special category patients, drug interactions and finally the management of adverse drug reactions in the mouth and associated structures. Within each chapter the authors describe both the adverse effects of drugs on individual oro-facial structures and the potential adverse reactions of different groups of drugs. Each chapter is followed by an extensive bibliography of the most recent references and an appendix serves to summarize the drugs most commonly implicated in producing oro-facial changes. In addition to describing adverse reactions there has been careful attention paid to summarizing the likely pathological mechanisms involved which enhances considerably the reader's interest in the subject. The index could be improved somewhat in that although there is reference in the text to amalgam (mercury) and toothpaste reactions, neither is cited separately in the subject index.

In summary, this is an excellent book and a very worthwhile addition to any doctor's or dentist's collection.

P.LAMEY

News and Notices

British Photodermatology Group (BPG)

A new group has been formed to act as a forum for all those with experience or interest in clinical and investigative photodermatology. The field covers many disciplines from molecular photophysics to clinical therapeutics. The primary aim of the BPG is to promote active communication and collaboration between researchers whose expertise may contribute to increased understanding of mechanisms of normal and abnormal cutaneous reactions to light, improve diagnosis and treatment of the photodermatoses, and optimize the efficacy and safety of the photo(chemo)therapies.

The first meeting will be at the summer meeting of the British Association of Dermatologists on the afternoon of Friday 7 July 1989 at Addenbrooke's Hospital, Cambridge. This session will comprise invited speakers and short oral communications. Those wishing to present a paper on any subject relevant to photodermatology should send a typed abstract of 250-300 words to the Secretary.

For further information contact the Secretary of the BPG: Dr Neil K. Gibbs, Photobiology Unit, Dermatology Dept, Ninewells Hospital, Dundee DD1 9SY, U.K.

Third Congress of the European Society of Pediatric Dermatology Bordeaux, France, 21-23 September 1990

The third Congress of the European Society of Pediatric Dermatology (ESPD) will be held in Bordeaux, 21-23 September 1990. The Congress will be held in English and French (with simultaneous translation), and the topics covered will include neonatal dermatology, genodermatoses, seborrhoeic dermatitis, atopic dermatitis, bullous diseases, connective tissue diseases, infectious dermatosis, tumours, STD and child abuse, and newer therapies in pediatric dermatology.

For further details contact: Professor J. Maleville, Service de Dermatologie Pédiatrique, Hôpital des Enfants, 168 cours de l'Argonne, F 33077 Bordeaux Cedex, France.

Course on Occupational and Contact Dermatitis University of Birmingham, 30 October-3 November 1989

A 5 day residential course on occupational and contact dermatitis is to be held at the Institute of Occupational Health, University of Birmingham and the Skin Hospital, Birmingham, from Monday 30 October until Friday 3 November 1989. Further details may be obtained from Joyce Blake, Course Co-ordinator, Institute of Occupational Health, University of Birmingham, PO Box 363, Birmingham B15 2TT.

35th General Assembly of the International Union against Venereal Diseases and the Treponematoses 9-11 May 1990

A meeting will be held at the Royal Society of Medicine in London on Sexually transmitted diseases in the age of AIDS. Further details can be obtained from Barbara Komoniewska BA, The Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE.

International Society for Bioengineering of the Skin Copenhagen, 15 June 1989

A 1-day meeting will be held on Rationales behind Moisturisers in Dermatology at the Rigshospitalet in Copenhagen on Thursday 15 June 1989. This will follow the 25th Scandinavian Dermatology Meeting. For further information contact Jørgen Serup MB, PhD, Rigshospitalet, Department of Dermatology H 5132, 9 Blegdamsvej, 2100 Copenhagen, Denmark.

**International Congress on Sexually Transmitted Diseases: Viral infections as cause of STD
Amsterdam 17-18 November 1989**

An International Congress on sexually transmitted diseases and viral infections as a cause of STD will be held in Amsterdam on 17 and 18 November 1989. Specific topics that will be covered will be on human papilloma virus infections, herpes simplex, hepatitis and human immunodeficiency virus infection. For further details contact: Hoboken Congress Organisation, Erasmus University, Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. Tel: 31 (0) 10-408 7880/7881/7-82.

Josie Bradbury Travel Award 1989

Applications are invited for this award from suitable applicants interested in the study of any aspect of psoriasis and its treatment. Financial grants may be made towards expenses incurred by any medical practitioners, research scientists, nurses, social workers, or members of any other profession relevant to the understanding and treatment of psoriasis. Applications should be made to: Mrs L.A.Henley, National Secretary, The Psoriasis Association, 7 Milton Street, Northampton NN2 7JG. Tel: 0604-711129.

The Skin Disease Research Fund

The Fund is pleased to invite further applications for grants. The deadline for submission is 4 September 1989 and it is expected that an indication of the result will be given during November. Only the Fund's own forms will be accepted and these may be obtained from the Hon. Appeal Secretary, D.K.G.Gowers, 12 Knighton Close, South Croydon, Surrey CR2 6DP. (Tel: 01-668 4300).

Index

SUBJECTS

- Acanthosis nigricans and Becker's naevus (Correspondence), 716
- Acne and abnormal androgens, 649
- Acne and polycystic ovaries (Paper summary), 293
- Acral pustulosis, 107
- Acrokeratotic poikiloderma of the Kindler-Weary type (Summary) 127
- Actinic prurigo treated with PUVA, 411
- Actinic reticuloid immunocytology, 779
- Alopecia totalis, treatment with allergic and irritant contact dermatitis compared, 397
- Angioedema vibratory, 93
- Anticardiolipin antibodies and livedo reticularis, 215
- Anti-melanoma antibody analysis (Paper summary), 290
- Apocrine sweat glands and androgen metabolism (Paper summary), 294
- Arteritis giant cell temporal, 843
- Atopic dermatitis and house dust mites, 245
- Atopic dermatitis and premenstrual exacerbation (Correspondence), 715
- Atopic dermatitis mononuclear leukocyte PDE activity, 607
- Atopic eczema and house dust mite (Paper summary), 298
- Atopic eczema and intestinal permeability, 71
- Atopic skin and platelet activating factor (PAF-acether), 287
- Barrier creams evaluated, 665
- Basal cell xenografts in mice, 351
- Becker's naevus and acanthosis nigricans (Correspondence), 716
- Behcet's disease treated with 5-amino-salicylic acid (Correspondence), 471
- Benzo(a)pyrene metabolism in cultured fibroblasts and multiple basal cell carcinoma, 161
- Bone effects and retinoid therapy, 31
- Book reviews:
- Advanced Geriatric Medicine 6*. Edited by F.I. CAIRD and J. GRIMLEY EVANS, 720
- Advances in Immunohistochemistry*. Edited by R.A. DELLIS, 860
- Adverse Drug Reactions in Dentistry*. R.A. SEYMOUR and J.G. WALTON, 860
- Computing for Clinicians*. T. CHARD, 476
- Das AIDS virus*. KARIN MÖLLING, 476
- Difficult Diagnoses in Dermatology*. Edited by M. LEBWOHL, 475
- Diseases of the Veins, Pathology, Diagnosis and Treatment*. N.L. BROWSE, K.G. BURNAND and M.L. THOMAS, 719
- Essential Paediatric Dermatology*. J. VERBOV, 859
- Hair Transplantation*. Edited by W.P. UNGER and R.E.A. NORDSTROM, 475
- Kaposi's Sarcoma: A Text and Atlas*. Edited by G. GOTTLIEB and B. ACKERMAN, 477
- Skin Cancer: Recognition and Management*. Edited by R.S. SCHWARZ, 720
- Surgical Application of Lasers* (2nd Edition) Edited by J.A. DIXON, 476
- The Melanotropins: Chemistry, Physiology and Mechanisms of Action*. A.N. EBERLE, 475
- Textbook of Haematology*. SHIRLYN B. MCKENZIE, 719
- Vascular Birthmarks: Haemangiomas and Malformations*. J.B. MULLIKEN and A.E. YOUNG, 859
- The Vulva*. Edited by C.M. RIDLEY, 720
- Vulvovaginal Candidosis—Theory and Practice*. W. MENDLING, 327
- Year Book of Dermatology* (1988), 859
- Bullous acrokeratotic poikiloderma, Kindler-Weary type (Case report), 147
- Bullous pemphigoid and dapsone, 82
- Bullous pemphigoid and pemphigus vulgaris sera bound to SCaBER cell line in an ELISA, 59
- Cataracts and long-term topical steroids (Correspondence), 472
- Chilblains treated with nifedipine, 267
- Chondrodysplasia punctata X-linked dominant (10 year follow-up), (Case report) 128
- Colitis with intra-epidermal neutrophilic IgA dermatosis, 113
- Congenital ichthyoses, enzymatic diagnosis (Summary), 135
- Cryptococcosis treated with oral fluconazole, 683
- Cutaneous chylous reflux, 695
- Cyclosporin and lymphocyte chemotaxis (Paper summary), 297
- Cyclosporin for psoriasis, 253
- Cyclosporin intralesionally in psoriasis, 207
- Dapsone for bullous pemphigoid, 83
- Darier-like solar keratosis, 383
- Darier's disease with depigmentation, 827
- Delayed pressure urticaria, 403
- Dermatophilus congolensis*, host response (Paper summary), 298
- Dermatophyte infections, antigen recognition (Paper summary), 299
- Desmosomal components and SDS envelopes (Paper summary), 299
- Dinitrochlorobenzene hypersensitivity, sex differences, 371

- Dinitrochlorobenzene sensitivity, sex differences (Paper summary), 297
- Dithranol therapy, short contact, 277
- Dowling Degos disease and Kitamura's reticulate acropigmentation, 463
- Dust-and rod-like particles and systemic lupus erythematosus (Correspondence), 851
- Dyskeratoses and α -keratin compositions, 363
- Elastic stockings, paradoxical effects (Summary), 133
- Epidermal basement membrane in BCC (Poster), 304
- Epidermal basement membrane zone antigens (Poster), 304
- Epidermal hyperkeratosis and malignancies (Correspondence), 141
- Epithelioid cell histiocytoma: a new entity, 185
- Erratum, 723
- Erythema multiforme due to griseofulvin, 455
- Exogenous ochronosis, 229
- Familial keratoses and hypersensitivity to UVA, 671
- Filament formation from α -keratins (Poster), 307
- Fish handlers and warts, 375
- Gamma-interferon and keratinocyte antigens, 1
- GB1-monoclonal antibody and common antigen expression (Poster), 305
- Genetic mosaics in dermatology (Summary), 134
- Giant cell (temporal) arteritis, 843
- Gravitational syndrome, thickness and extensibility (Poster), 311
- Great toe-nail dystrophy (Correspondence), 139
- Gynaecomastia and etretinate (Correspondence), 317
- Haemophiliacs with skin disease, 795
- Hair surface architecture using water as mounting medium (Correspondence), 717
- Hand eczema, occupational and non-occupational compared, 77
- Histamine release of prostacyclin from endothelial cells, 287
- Histiocytoma cutis: a tumour of dermal dendrocytes (dermal dendrocytoma), 197
- House dust mites and atopic dermatitis, 245
- Human keratinocytes and gene transcription (Paper summary), 303
- Human sweat, free fatty acids and sterols, 43
- Human T-cell proliferation dependent on purity of cells used (Paper summary), 301
- Hyper IgE syndrome and generalized molluscum contagiosum (Case report), 125
- Hypotrichosis of the Marie Unna type (Case report), 129
- Ichthyosis, enzymatic diagnosis (Summary), 135
- IgA dermatosis with colitis, 113
- Immunotherapy topical (Summary), 137
- Inflammatory cutaneous disease and OKM₁ antigen (Paper summary), 389
- Infundibular tumours of the head and neck, 633
- Interferon-gamma and normal human skin (Paper summary), 301
- Interleukin-1 from human stratum corneum (Paper summary), 288
- Interleukin-1 α and release of prostacyclin from endothelial cells, 153
- Intestinal permeability in atopic eczema, 71
- Intracellular calcium binding protein in vesico-bullous disorders (Paper summary), 289
- Intradermal lignocaine and fibroblasts (Poster), 308
- Isotretinoin and CRABP, 639
- Kaposi's sarcoma regressing on Zidovudine in AIDS, 709
- α -keratin compositions in dyskeratoses, 363
- Keratosis palmoplantaris transgrediens et progrediens Greither (Case report), 125
- Ketanserin in systemic sclerosis, 261
- Langerhans cells and low-protein diet, 341
- Langerhans cells and lymphocyte subsets in chronic renal failure, 745
- Langerhans cells blood dendrites and monocytes compared (Paper summary), 300
- Langerhans cells and PUVA therapy, 329
- Langerhans cells in oral lichen planus, 223
- Langerhans cells, UVB induced damage (Poster), 309
- Laser surgery and rare-earth doped eyewear (Correspondence), 470
- Leg ulceration with livedoid vasculitis and response to ketanserin, 101
- Leg ulcers and ultraviolet light, 809
- Leukoplakia and Langerhans cells, 223
- Leukotriene B₄ photodegradation by UVB and UVA irradiation, 145
- Lichen planus and captopril (Correspondence), 319
- Lipids in eccrine sweat, 49
- Lipomatosis and familial hyperlipidaemia, 689
- Livedo reticularis and anticardiolipin antibodies, 215
- Lupus anticoagulant and associated skin lesions, 419
- Lupus erythematosus and anticardiolipin syndrome (Correspondence), 469
- Lymphocytes and gamma-interferon (Paper summary), 302
- Lymphocytic lymphoma and C₁ esterase inhibitor deficiency, 121
- Malignant potential of melanocytic naevi, 137
- Mast cells, staining, 625
- Mast cell variation (Poster), 303
- Mediators in delayed pressure urticaria (Poster), 310
- Melanocytic lesions immunohistochemistry (Summary), 137
- Melanogenesis and vitamin D₃, 21
- B16 melanoma cells, adhesion characteristics (Poster), 309
- Methotrexate in severe psoriasis (Poster), 306
- Meyerson's naevi in pityriasis rosea (Correspondence), 318
- Molluscum contagiosum virus types in genital and non-genital lesions, 37
- Morphoea and autoimmune disorders, 645
- Morphoea, post-irradiation, 831
- Morphoea profunda, solitary, 431
- Naevoid psoriasis, 837
- News and Notices, 327, 478, 587, 722, 861
- Nifedipine for chilblains, 267

- NK cell activity and CTCL prognosis (Paper summary), 294
- Obituaries:
 Dr Owen Finn, 315
 Dr Louis Forman, 847
 Dr H.W. Gordon, 467
- Ochronosis, exogenous, 229
- Oestrogen receptors in congenital naevi (Posters), 308
- OKM5 antigen expression in cutaneous inflammation, 613
- Ossification, congenital cutaneous (Case report), 132
- Palmo-plantar pustulosis and cyclosporin (Correspondence), 857
- Pemphigoid vegetans, 449
- Pemphigoid gestationis, placental immunopathology, 735
- Pemphigus, immunofluorescent tests in remission, 391
- Phospholipid esterified octadeca-9 cis, 11 trans-dienoic acid. (Paper summary), 290
- Phototoxicity quinolone induced (Paper summary), 291
- Pili torti, dystrophic (Correspondence), 323
- Pityrosporum ovale* and infantile seborrhoeic dermatitis, 359
- Plasminogen and plasminogen activators in human skin (Poster), 307
- Platelet hyperaggregability in Klinefelter's syndrome (Correspondence), 322
- Polymorphic light eruption: immunopathology, 173
- Porphyria, urine screening (Poster), 313
- Prostacyclin release from endothelial cells, 737
- Proteus syndrome (Case report), 129
- Prurigo nodularis and calcitonin gene-related peptide, 619
- Prurigo pigmentosa and minocycline, 705
- Pseudoephedrine fixed drug reaction (Correspondence), 858
- Psoralen-UVA and UVB erythema dose response curves (Paper summary), 292
- Psoriasis, acetrelin and UVB compared, 665
- Psoriasis and arthritis, DNA variants (Paper summary), 288
- Psoriasis and long-term cyclosporin, 253
- Psoriasis, copper levels, serum ceruloplasmin, superoxide dismutase and myeloperoxidase activity, 239
- Psoriasis, fish oil and low-dose UVB, 801
- Psoriasis, intralesional cyclosporin, 207
- Psoriasis, mononuclear leukocyte cyclic AMP phosphodiesterase activity (Paper summary), 285
- Psoriasis, naevoid, 837
- Psoriasis, palmoplantar pustular psoriasis is a distinct disease entity (Paper summary), 286
- Psoriasis plaque areas compared (Paper summary), 293
- Psoriasis research, what is new? (Summary), 133
- Psoriasis, serum IL-2 receptor levels (Paper summary), 285
- Psoriasis, topical 1 α , 25-dihydroxyvitamin D₃, 661
- Psoriasis topical nordihydroguaiaretic acid (NDGA), 286
- Psoriatic plaques, keratin gene expression, 9
- Psoriatic plaques, the active edge (Poster), 606
- Psoriatic skin and oral etretinate (Poster), 305
- Purpura and sepsis (Correspondence), 715
- PUVA for actinic prurigo, 411
- PUVA therapy and Langerhans cells, 329
- PUVA therapy decreases Langerhans cells (Paper summary), 300
- Raynaud's phenomenon and topical hexyl nicotinate lotion (Poster), 311
- Resistance to azelaic acid (Poster), 313
- Retinoid therapy and bone changes, 31
- Rickettsial African spotted fever, 701
- 3, St. Andrew's Place, Seventy years on, 473
- Schöpf syndrome (Case report), 131
- Seborrhoeic dermatitis and sebum reduction (Paper summary), 294
- Seborrhoeic dermatitis in infants and *Pityrosporum ovale*, 359
- Sepsis and purpura (Correspondence), 715
- Skin blood flow measured by Doppler (Paper summary), 282
- Skin lesions with toxic gases (Summary), 136
- Sneddon's syndrome with livedo reticularis and anti-cardiolipin-antibodies, 441
- Solar keratosis, acantholysis and preneoplasia, 383
- Stereoisomers of 12-HETE in human epidermis, 49
- Superoxide dismutase and myeloperoxidase activity, serum ceruloplasmin and copper levels in psoriasis, 239
- Sweat, free fatty acids and sterols, 43
- Systemic sclerosis and ketanserin, 261
- Systemic sclerosis, epidermal class II antigen expression and Langerhans cell density (Poster), 312
- T-cell lymphomas and DNA analysis, 597
- Tattoos treated with carbon dioxide laser, 819
- Trichophyton mentagrophytes* colonization (Poster), 313
- Trimethylaminuria, an unusual case, 459
- Urticaria and anaphylatoxin levels (Poster), 310
- Urticaria, delayed pressure, 403
- Urticaria, transfer of a serum factor from human to monkey (Correspondence), 853
- Urticaria treated by desensitization with grass pollen extract, 409
- UVA sunbeds, 767
- UVB-induced photo immunosuppression, 589
- Vibratory angioedema, 93
- Virally transformed human keratinocytes (Paper summary), 303
- Vitamin D₃ and melanogenesis, 21
- Vitiligo following diphenycprone dermatitis (Correspondence), 323
- Vitronectin in complement activating skin disorders, 725
- Warts in fish handlers, 375
- Zidovudine and oral Kaposi's sarcoma, 709

AUTHORS

- ABDULLAH, A.N., KECZKES, K. and WYATT, E.H. Skin necrosis in giant cell (temporal) arteritis: report of three cases, 843
- ALEGRE, V.A., GASTINEAU, D.A. and WINKELMANN, R.K. Skin lesions associated with circulating lupus anticoagulant, 419
- ALJABRE, S.J., SCOTT, E.M. and RICHARDSON, M.D. Early events in the colonization and invasion of stratum corneum by *Trichophyton mentagrophytes* (Poster), 313
- ALLEN, M.H., BARKER, J.N.W.N., MARKEY, A.C. and MACDONALD, D.M. Expression of OKM₅ antigen on keratinocytes in inflammatory cutaneous disease (Paper summary), 289
- ALLEN, M.H. *see* BARKER, J.N.W.N.
- ANDERSON, T.F. *see* GUPTA, A.K.
- ANDRAUS, M.C. *see* BARBA, A.
- ARCHARD, L.C. *see* PORTER, C.D.
- ARCHER, C.B. *see* MARKEY, A.C.
- ARGENBRIGHT, L.W. *see* OPAS, E.E.
- ASHERSON, R.A., MAYOU, S.C., MERRY, P., BLACK, M.M. and HUGHES, G.R.V. The spectrum of livedo reticularis and anticardiolipin antibodies, 215
- ASHWORTH, J., KAHAN, M.C. and BREATHNACH, S.M. PUVA therapy decreases HLA-DR⁺T6⁺ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA-DR⁺T6⁺ Langerhans cells exhibit normal alloantigen-presenting function (Paper summary), 300
- ASHWORTH, J., KAHAN, M.C. and BREATHNACH, S.M. PUVA therapy decreases HLA-DR⁺ CD1a⁺ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA-DR⁺ CD1a⁺ Langerhans cells exhibit normal alloantigen-presenting function, 329
- ASHWORTH, J., TUYP, E. and MACKIE, R.M. Allergic and irritant contact dermatitis compared in the treatment of alopecia totalis and universalis. A comparison of the value of topical diphenycprone and tretinoin gel, 397
- ASHWORTH, J., WICKENS, D.G., MAURICE, P.D., LEWIS, G., DORMAND, T.L. and BREATHNACH, S.M. Phospholipid esterified octadeca-9 cis, 11 trans-denoic acid (a measure of free radical formation) in benign, premalignant and malignant skin lesions (Paper summary), 290
- ASO, M., MIYAMOTO, T., MORIMURA, T. and SHIMAO, S. Prurigo pigmentosa successfully treated with minocycline, 705
- ATHERTON, D.J., BOTCHERBY, P.K., FRANCIS, A.J., SMITH, N.P., WELLS, R.S. and GIANNELLI, F. Familial keratosis of actinic distribution associated with internal malignancy and cellular hypersensitivity to UVA, 671
- ATHERTON, D.J., KAHANA, M. and RUSSELL-JONES, R. Naevoid psoriasis, 837
- AUGUST, P.J. and O'DRISCOLL, J. Urticaria successfully treated by desensitization with grass pollen extract, 409
- AYATOLLAHI, A. *see* PAYNE, P.A.
- BACON, K.B. *see* ROSS, J.S.
- BAINS, R. *see* RUSTIN, M.H.A.
- BAKER, B.S., POWLES, A.V., SAVAGE, C.R., MCFADDEN, J.P., VALDIMARSSON, H. and FRY, L. Intralesional cyclosporin in psoriasis: effects in T lymphocytes and dendritic cell subpopulations, 207
- BAKER, B.S. *see* GRIFFITHS, C.E.M.
- BANNERJEE, S. *see* EDWARDS, J.M.
- BARAN, R. Great toe-nail dystrophy (Correspondence), 139
- BARBA, A., SCHENA, D., ANDRAUS, M.C., FACCINI, G., PASINI, F., BROCCO, G., CAVALLINI, G., SCURO, A. and CHIEREGATO, G.C. Intestinal permeability in patients with atopic eczema, 71
- BARKER, J.N.W.N. *see* MARKEY, A.C.
- BARKER, J.N.W.N. and MACDONALD, D.M. *In vivo* effects of interferon-gamma on normal human skin (Paper summary), 301
- BARKER, J.N.W.N., MARKEY, A.C., ALLEN, M.H. and MACDONALD, D.M. Keratinocyte expression of OKM₅ antigen in inflammatory cutaneous disease, 613
- BARKER, J.N.W.N. *see* ALLEN, M.H.
- BARKER, J.N.W.N. *see* KERR, L.A.P.
- BARKER, J.N.W.N. *see* NAVSARIA, H.
- BARR R.M., *see* LAWLER, F.
- BARR, R. *see* NEWTON, J.A.
- BARTH, J.H. and KEALEY, T. Androgen metabolism by isolated human apocrine sweat glands (Paper summary), 294
- BEARD, J. *see* HIGGINS, E.J.
- BECK, H.I. and KORSGAARD, J. Atopic dermatitis and house dust mites, 245
- BEN-CHETRIT, A. *see* DAVID, M.
- BERARDESCA, E. and MAIBACH, H. Cutaneous reactive hyperaemia: racial differences induced by corticoid application, 787
- BERGER, N.A. *see* DON, P.S.C.
- BERTH-JONES, J. and GRAHAM-BROWN, R.A.C. A family with Dowling Degos disease showing features of Kitamura's reticulate acropigmentation, 434
- BERTH-JONES, J. and HUTCHINSON, P.E. Darier's disease and peri-follicular depigmentation, 827
- BERTH-JONES, J., HUTCHINSON, P.E., WICKS, A.C.B., WARREN, J. and MITCHELL, V.E. Discoid lupus erythematosus associated with the anticardiolipin syndrome (Correspondence), 469
- BIANCHI, P.A. *see* RANZI, T.
- BICKERS, D.R. *see* DON, P.S.C.
- BIRD, C.R. *see* CAMP, R.D.R.
- BLACK, M.M. *see* ASHERSON, R.A.

- BLACK, M.M. *see* KELLY, S.E.
 BLAKE, N.W. *see* PORTER, C.D.
 BLEEHEN, S.S. *see* HILL, S.E.
 BOER, J. *see* JEST, J.
 BOJAR, R.A. *see* HOLLAND, K.T.
 BOODLE, K.M. *see* NEWTON, J.A.
 BOTCHERBY, P.K. *see* ATHERTON, D.J.
 BOUMAN, T. Bullous acrokeratotic poikiloderma of the Kindler-Weary type (Case report), 127
 BRAKKE, A.J.M. *see* KUIJER, K.P.
 BREATHNACH, A.S. *see* LAWLOR, F.
 BREATHNACH, S.M. *see* ASHWORTH, J.
 BROBERG, A. and FAERGEMANN, J. Infantile seborrhoeic dermatitis and *Pityrosporum ovale*, 359
 BROCCO, G. *see* BARBA, A.
 BRÖCKER, E.B. *see* STEIJLEN, P.
 BRUCE, S. *see* HOFFMANN, T.J.
 BULL, H.A., RUSTIN, M.H.A. and DOWD, P.M. Histamine-induced release of prostacyclin from human endothelial cells (Paper summary), 287
 BULL, H.A., RUSTIN, M.H.A. and DOWD, P.M. Release of prostacyclin from cultured human endothelial cells following prolonged exposure to histamine, 757
 BULL, H.A. *see* RUSTIN, M.H.A.
 BUNKER, C.B., NEWTON, J.A., KILBORN, J., PATEL, A., CONWAY, G.S., JACOBS, H.S., GREAVES, M.W. and DOWD, P.M. Women with acne who have polycystic ovaries (PCO) have endocrinological differences from other women with PCO (Paper summary), 293
 BUNKER, C.B. *see* DOWD, P.M.
 BUNKER, C.B. *see* RUSTIN, M.H.A.
 BUNNEY, M.H. *see* RÜDLINGER, R.
 BURGE, S.M., CEDERHOLM-WILLIAMS, S. and RYAN, T.J. Plasminogen and plasminogen activators in human skin in health and disease (Poster), 307
 BURKE, B. *see* LEVILL, M.J.
 BURTON, J.L. *see* GRATTAN, C.E.H.
 BUTLER, P.H. *see* VAN HALEWYN C.N.
 CABILI, S. *see* RUBINSTEIN, A.
 CAMISA, C. Fixed drug reactions to pseudoephedrine hydrochloride (Correspondence), 858
 CAMP, R.D.R., FINCHAM, N.J., ROSS, J.S. and BIRD, C.R. Characterization and pro-inflammatory activity in human skin of interleukin-1 (IL-1)-like material extracted from normal stratum corneum (Paper summary), 288
 CAMP, R.D.R. *see* ROSS, J.S.
 CAMPANINI, M. *see* RANZI, T.
 CARMICHAEL, A.J. and PAUL, C.J. Reversible gynaecomastia associated with etretinate (Correspondence), 317
 CARTWRIGHT, P.H., ILDERTON, E., SOWDEN, J.M., YARDLEY, H.J. Further evidence from measurement of epidermal phospholipase A₂ activity that chronic palmoplantar pustular psoriasis is a disease entity distinct from psoriasis vulgaris, 286
 CATI-GIOVANNELLI, B. *see* COSTAGLIOLA, C.
 CAVALLINI, G. *see* BARBA, A.
 CAWOOD, M.L. *see* LEVILL, M.J.
 CEDERHOLM-WILLIAMS, S. *see* BURGE, S.M.
 CERIO, R., SPAULI, J. and WILSON JONES, E. Histiocytoma cutis: a tumour of dermal dendrocytes (dermal dendrocytoma), 197
 CERIO, R. *see* WILSON JONES, E.
 CHALMERS, R.J.G. *see* MUSTAFA, Z.H.AL-
 CHERN, L.-C., LIN, C.-S. and WONG, C.-K. Cutaneous chylous reflux, 695
 CHIEREGATO, G.C. *see* BARBA, A.
 CHRISTENSEN, J.B.J. *see* RALFKIAER, E.
 CHU, A., MORRIS, J. and SMITH, N. NK cell activity as a prognostic factor in CTCL-prospective 3-year study (Paper summary), 294
 CHU, A.C. *see* MCLELLAND, J.
 CHU, A.C. *see* NORRIS, P.G.
 CHU, A.C. *see* RIVERS, J.K.
 CHURCHILL, L.J., MARKEY, A.C. and MACDONALD, D.M. Variation of *in situ* mast cell populations using a range of histochemical techniques (Poster), 303
 CHURCHILL, L.J. *see* MARKEY, A.C.
 CLARKE, C.W.F. *see* RICHARDSON, S.G.N.
 COLVER, G.B., RODGER, A., MORTIMER, P.S., SAVIN, J.A., NEILL, S.M. and HUNTER, J.A.A. Post-irradiation morphoea, 831
 COLVER, G.B. *see* KEMMETT, D.
 CONNOLLY, C.N. *see* ROWLAND-PAYNE, C.M.E.
 CONWAY, G.S. *see* BUNKER, C.B.
 COOK, A.L., MALLETT, R.B., HANAHOE, T.H.P. and PYE, R.J. Binding of bullous pemphigoid and pemphigus vulgaris sera to SCaBER cell line in an ELISA, 59
 COOKE, K.B. *see* MAJID, A.
 COOPER, M.A.C.S. *see* EDWARDS, J.M.
 COPEMAN, P.W.M. *see* MAJID, A.
 COSTAGLIOLA, C., CATI-GIOVANNELLI, B., PICCIRILLO, A. and DELFINO, M. Cataracts associated with long-term topical steroids (Correspondence), 472
 COTTERILL, J.A. *see* LANIGAN, S.W.
 COTTERILL, J.A.C. *see* MACDONALD-HULL, S.P.
 COULSON, I.H., DUNCAN, S.N. and HOLDEN, C.A. Peripheral blood mononuclear leukocyte cyclic adenosine monophosphate specific phosphodiesterase activity in childhood atopic eczema, 607
 COULSON, I.H. *see* HURT, G.R.
 COWLEY, N.C., FARR, P.M. and SHUSTER, S. The effect of sebum reduction on seborrhoeic dermatitis (Paper summary), 294
 COX, N.H., FARR, P.M. and DIFFEY, B.L. Comparison of dose-response curves for psoralen-UVA and UVB erythema (Paper summary), 292
 COX, N.H., TAPSON, N.S. and FARR, P.M. Lichen planus associated with captopril (Correspondence), 319
 CREAM, J.J. *see* PORTER, C.D.
 CROMWELL, O. *see* LAWLER, F.
 CROVATO, F., NAZZARI, G., GAMBINI, C. and MASSONE, L. Meyerson's naevi in pityriasis rosea (Correspondence), 318
 CUNLIFFE, W.J. *see* HOLLAND, D.B.
 CUNLIFFE, W.J. *see* HOLLAND, K.T.
 CUNLIFFE, W.J. *see* LEVILL, M.J.
 CUNLIFFE, W.J. *see* MACDONALD-HULL, S.
 CUTLER, T.P. *see* TELFER, N.R.
 CZARNETZKI, B.M. *see* VAN DE KERKHOF, P.C.M.
 DAHLBÄCK, K., LÖFBERG, H. and DAHLBÄCK, B. Vitronectin localizes with Ig deposits and C9 neoantigen in

- discoid lupus erythematosus and dermatitis herpetiformis, but not in bullous pemphigoid, 725
- DAHLBÄCK, B. *see* DAHLBÄCK, K.
- DALY, H.M. *see* MUNRO, C.S.
- DARE, R.A.S. *see* LANIGAN, S.W.
- DAS, M. *see* DON, P.S.C.
- DAVID, M., WEISSMAN-KATZENELSON, V., BENCHETRIT, A., HAZAZ, B., INGBER, A. and SANDBANK, M. The usefulness of immunofluorescent tests in pemphigus patients in clinical remission, 391
- DAWBER, R.P.R. *see* TELFER, N.R.
- DAWSON, T.A.J. Great toe-nail dystrophy (Correspondence), 139
- DELFINO, M. *see* COSTAGLIOLA, C.
- DEMAINE, A. *see* SAKKAS, L.I.
- DIFFEY, B.L. *see* COX, N.H.
- DIFFEY, B.L. *see* FARR, P.M.
- DODD, H.J., SARKANY, I. and GAYLARDE, P.M. The short-term benefit and long-term failure of ultraviolet light in the treatment of venous leg ulcers, 809
- DOĞAN, P., SOYUER, Ü. and TANRIKULU, G. Superoxide dismutase and myeloperoxidase activity in polymorphonuclear leukocytes, and serum ceruplasmin and copper levels in psoriasis, 239
- DON, P.S.C., MUKHTAR, H., DAS, M., BERGER, N.A. and BICKERS, D.R. Benzo(a) metabolism, DNA-binding and UV-induced repair of DNA damage in cultured skin fibroblasts from a patient with unilateral multiple basal cell carcinoma, 161
- DORMAND, T.L. *see* ASHWORTH, J.
- DOWD, P.M., LANIGAN, S., RUSTIN, M.H.A. and BUNKER, C.B. Topically applied hexyl nicotinate lotion increases the cutaneous blood flow in patients with Raynaud's phenomenon (Poster), 311
- DOWD, P.M. *see* BULL, H.A.
- DOWD, P.M. *see* BUNKER, C.B.
- DOWD, P.M. *see* NEWTON, J.A.
- DOWD, P.M. *see* RUSTIN, M.H.A.
- DUFF, G.W. *see* KEMMETT, D.
- DUJARDIN, P. *see* ORTONNE, J.P.
- DUNCAN, S.N. *see* COULSON, I.H.
- DUNSMORE, I.R. *see* HARRINGTON, C.I.
- DYKES, P.J. *see* MARKS, R.
- EAD, R. *see* GEBRIL, M.G.
- EADY, R.A.J. *see* SCHOFIELD, O.M.V.
- EADY, R.A.J. *see* SHIMIZU, H.
- EDWARD, M., HAMILTON, M. and MACKIE, R.M. Adhesion characteristics of metastatic and non-metastatic B16 melanoma cells *in vitro* (Poster), 309
- EDWARDS, C. *see* MOURAD, M.M.
- EDWARDS, J.M., COOPER, M.A.C.S. and BANNERJEE, S. Congenital epidermolytic hyperkeratosis associated with multiple malignancies (Correspondence), 141
- ELLIS, C.N. *see* GUPTA, A.K.
- EVANS, M. *see* HALES, S.A.
- FACCINI, G. *see* BARBA, A.
- FAERGEMANN, J. *see* BROBERG, A.
- FARR, P.M. and DIFFEY, B.L. Treatment of actinic prurigo with PUVA: mechanism of action, 411
- FARR, P.M. *see* COWLEY, N.C.
- FARR, P.M. *see* COX, N.H.
- FERGUSON, J., WALKER, E.M. and JOHNSON, B.E. Quinolone antibiotic-induced phototoxicity (Paper summary), 291
- FERGUSON, J. *see* MILLAR, B.
- FINCHAM, N.J. *see* CAMP, R.D.R.
- FINDLAY, G.H. Multiple infundibular tumours of the head and neck, 633
- FLEMING, K.A. *see* HALES, S.A.
- FLEMING, S. *see* KELLY, S.E.
- FLEUREN, W.A. and WEEMAES, C. The hyper IgE syndrome and generalized molluscum contagiosum (Case report), 125
- FLORIO, M. *see* RUOCO, V.
- FRITAG, B. *see* ORTONNE, J.P.
- FRANCIS, A.R. *see* ATHERTON, D.J.
- FREDJ-REYGROBELLET, B. *see* SCHOFIELD, O.M.V.
- FRIEDMANN, P.S. *see* REES, J.L.
- FRIEDMANN, P.S. *see* MUNRO, C.S.
- FRY, L. *see* BAKER, B.S.
- FRY, L. *see* GRIFFITHS, C.E.M.
- GALUSKA, L. *see* TÖRÖK, L.
- GAMBINI, C. *see* CROVATO, F.
- GASSON, G.B. *see* RICHARDSON, S.G.N.
- GASTINEAU, D.A. *see* ALEGRE, V.A.
- GAYLARDE, P.M. *see* DODD, H.J.
- GAZIT, E. *see* RUBINSTEIN, A.
- GEBRIL, M.G., WINKOVE, C., McDOWELL, D., EAD, R., McDONALD, K. and SHEHADE, S. Chronic porphyria—improved urine screening (Poster) 313
- GHAZNAWIE, M., PAPADIMITRIOU, J.M. and HEENAN, P.J. Reduced density and morphologic alteration of Ia and ADPase positive Langerhans cells after low-protein diet, 341
- GHOHAL, B.S. *see* KELLY, S.E.
- GIANNELLI, F. *see* ATHERTON, D.J.
- GOH, C.L. An epidemiological comparison between occupational and non-occupational hand eczema, 77
- GOLD, S. Seventy years on: 3, St. Andrew's Place (Correspondence), 473
- GOODFIELD, M. *see* MACDONALD-HULL, S.
- GOOR, Y. *see* RUBINSTEIN, A.
- GRAHAM-BROWN, R.A.C. *see* BERTH-JONES, J.
- GRATTAN, C.E.H., BURTON, J.L. and BOON, A.P. Sneddon's syndrome (livedo reticularis and cerebral thrombosis) with livedo vasculitis and anticardiolipin antibodies, 441
- GRATTAN, C.E.H., WINKELMANN, R.K. and ZOLLMAN, P.E. Attempted passive transfer of a serum factor in chronic urticaria from human to monkey (Correspondence), 853
- GRATTAN, C.E.H. *see* HAMON, C.G.B.
- GREAVES, M.W. *see* BUNKER, C.B.
- GREAVES, M.W. *see* LAWLOR, F.
- GREAVES, M.W. *see* LAWLOR, F.
- GREAVES, M.W. *see* LAWLOR, F.
- GREAVES, M.W. *see* NEWTON, J.A.
- GREEN, C. *see* MILLAR, B.
- GRIFFITHS, C.E.M., POWLES, A.V., MCFADDEN, J., BAKER, B.S., VALDIMARSSON, H. and FRY, L. Long-term cyclosporin for psoriasis, 253
- GRIFFITHS, C.E.M., VOORHEES, J.J. and NICKOLOFF, B.J. Gamma-interferon induces different keratinocyte

- cellular patterns of expression of HLA-DR and DQ and inter-cellular adhesion molecule-1 (ICAM-1) antigens, 1
- GRIFFITHS, C.E.M., VOORHEES, J.J. and NICKOLOFF, B.J. Lymphocytes bind to the epidermis of frozen sections of normal skin treated with gamma-interferon (Paper summary), 302
- GRIMALDI FILIOLI F. *see* RUOCCO, V.
- GROB, R. *see* RÜDLINGER, R.
- GUERRERA, V. *see* RUOCCO, V.
- GUPTA, A.K., ELLIS, C.N., TELLNER, D.C., ANDERSON, T.F. and VOORHEES, J.J. Double-blind, placebo-controlled study to evaluate the efficacy of fish oil and low-dose UVB in the treatment of psoriasis, 801
- HABOUBI, N.Y. *see* MUSTAFA, Z.H.AL.
- HALES, S.A., STAMP, G., EVANS, M. and FLEMING, K.A. Identification of cells in human basal cell carcinoma xenografts in mice using *in situ* hybridization, 351
- HAMAMI, I. *see* MARKS, R.
- HAMBLIN, A. *see* PARKER, S.C.
- HAMILTON, M. *see* EDWARD, M.
- HAMM, H. *see* STEIJLEN, P.
- HAMON, C.G.B., GRATIAN, C.E.H. and LEEMING, R.J. Lack of correlation between serum anaphylatoxin levels and the skin test response to autologous serum in chronic urticaria (Poster), 310
- HANAHOB, T.H.P. *see* COOK, A.L.
- HAPPLE, R. Genetic mosaics in dermatology (Summary), 134
- HARDWICK, N., VAN GELDER, L.W., VAN DER MERWE, C.A. and VAN DER MERWE, M.P. Exogenous ochronosis: an epidemiological study, 229
- HARRINGTON, C.I. and DUNSMORE, L.R. An investigation into the incidence of auto-immune disorders in patients with localized morphea, 645
- HARRISON, P.V. *see* WALKER, G.B.
- HAWK, J.L.M. *see* NORRIS, P.G.
- HAWK, J.L.M. *see* RIVERS, J.K.
- HAWTHORN, R.J. *see* MCKERROW, K.J.
- HAY, R.J. *see* MOORE, M.K.
- HAZAZ, B. *see* DAVID, M.
- HEENAN, P.J. *see* GHANAWIE, M.
- HENQUET, C.J.M. *see* TOONSTRA, J.
- HIGGINS, E.J., TIDMAN, M.J., SAVIDGE, G.F., BEARD, J. and MACDONALD, D.M. Platelet hyperaggregability in two patients with Klinefelter's syndrome complicated by leg ulcers (Correspondence), 322
- HILL, S.E., BLEEHEN, S.S. and MACNEIL, S. 1 α -25-dihydroxyvitamin D₃ increases intracellular free calcium in murine B16 melanoma, 21
- HINNTER, H. *see* SEPP, N.
- HIRSCHEL-SCHOLZ, S., SIEGENTHALER, G. and SAURAT, J.-H. Isotretinoin differs from other synthetic retinoids in its modulation of human cellular retinoic acid binding protein (CRABP), 639
- HODGINS, M.B. *see* TILLMAN, D.M.
- HOFFMANN, T.J., KETTLER, A. and BRUCE, S. Acute acral pustulosis, 107
- HOLDEN, C.A. *see* COULSON, I.H.
- HOLDEN, C.A. *see* HURT, G.R.
- HOLDSWORTH, J. *see* VAN HALESWYN, C.N.
- HOLLAND, D.B., WOOD, E.J., CUNLIFFE, W.J. and TURNER, D.M. Keratin gene expression during the resolution of psoriatic plaques: effect of dithranol, PUVA, tretinoin and hydroxyurea regimens, 9
- HOLLAND, K.T., BOJAR, R.A. and CUNLIFFE, W.J. Can phenotypic and genotypic resistance to azelaic acid by cutaneous bacteria be promoted by long-term exposure to the compound (Poster), 313
- HSI, B.L. *see* SCHOFIELD, O.M.V.
- HUGHES, G.R.V. *see* ASHERSON, R.A.
- HULSMANS, F.-J.H. *see* HULSMANS, R.-F.H.
- HULSMANS, R.-F.H.J. and HULSMANS, F.-H. Concomitant Becker's naevus and acanthosis nigricans (Correspondence), 716
- HUMES, J.L. *see* OPAS, E.E.
- HUNTER, I. and SKERROW, D. Relationship of the final α -keratin compositions in dyskeratoses to those along the normal keratinization pathway: aetiological and diagnostic significance, 363
- HUNTER, J.A.A. *see* COLVER, G.B.
- HUNTER, J.A.A. *see* RÜDLINGER, R.
- HURT, G.R., COULSON, I.H. and HOLDEN, C.A. Mononuclear leukocyte cyclic AMP phosphodiesterase activity in psoriasis, 285
- HUTCHINSON, P.E. *see* BERTH-JONES, J.
- ILDERTON, E. *see* CARTWRIGHT, P.H.
- INGBER, A. *see* DAVID, M.
- ISHIBASHI, Y. *see* UEDA, Y.
- ISHII, N., KOMATSU, H., NAKAJIMA, H., TANI, K. and SUTO, T. Imported rickettsial African spotted fever in Japan, 701
- JACOBS, H.S. *see* BUNKER, C.B.
- JANSEN, C. *see* RÄSÄNEN, L.
- JEST, J. and BOER, J. Combined treatment of psoriasis with acitretin and UVB phototherapy compared with acitretin alone and UVB alone, 665
- JOHNSON, B.B. *see* FERGUSON, J.
- JOHNSON, J.P. *see* STEIJLEN, P.
- JONES, S.E. *see* KELLY, S.E.
- KÁDÁR, L. *see* TÖRÖK, L.
- KAHAN, M.C. *see* ASHWORTH, J.
- KAHANA, M. *see* ATHERTON, D.J.
- KÁSA, M. *see* TÖRÖK, L.
- KEALEY, T. *see* BARTH, J.H.
- KECZKES, K. *see* ABDULLAH, A.N.
- KELLY, S.E., FLEMING, S., GHOGAL, B.S., WOJNAROWSKA, F. and BLACK, M.M. Immunopathology of the placenta in pemphigoid gestationis and linear IgA disease, 735
- KELLY, S.E., JONES, D.B. and FLEMING, S.R. Epidermal expression of an intracellular calcium binding protein in vesiculo-bullous disorders (Paper summary), 289
- KEMMETT, D. Premenstrual exacerbation of atopic dermatitis (Correspondence), 715
- KEMMETT, D., SYMONDS, J.A., COLVER, G.B. and DUFF, G.W. Serum IL-2 receptor levels in patients with psoriasis (Papers), 285
- KENNEDY, A.R. *see* SHIMIZU, H.
- KERR, L.-A.P., BARKER, J.N.W.N., NAVSARIA, H.A., LEIGH, I.M., WELSH K.I. and MACDONALD, D.M.

- Interferon-gamma induced HLA-DR gene-transcription in human keratinocytes (Paper summary), 303
- KETTLER, A. *see* HOFFMANN, T.J.
- KILBORN, J. *see* BUNKER, C.B.
- KINN, S.R., SKERROW, C.J. and SKERROW, D. Incorporation of desmosomal components into an SDS-insoluble envelope *in vitro* (Paper summary), 299
- KLINGER, R. *see* WOLLONA, U.
- KNIGHT, R.A. *see* MAJID, A.
- KNIGHT, A.G. *see* SHUTTLEWORTH, D.
- KNOFF, B. *see* WOLLONA, U.
- KOBZA BLACK, A. *see* LAWLOR, F.
- KOMATSU, H. *see* ISHII, N.
- KOOPMAN, R. X-linked dominant chondrodysplasia punctata (10-year follow-up) (Case report), 128
- KORSGAARD, J. *see* BECK, H.I.
- KUIPER J.P. and BRAKKEE, A.J.M. The paradoxical effects of elastic stockings (Summary), 133
- KUNZE, R. *see* LANGFORD, A.
- LANGE WANTZIN G. *see* RALFKAER, E.
- LANGFORD, A., RUF, B., KUNZE R., POHLE, H.-D. and REICHAERT, P. Regression of oral Kaposi's sarcoma in a case of AIDS on Zidovudine (AZT), 709
- LANIGAN, S.W., DARE, R.A.S. and COTTERILL, J.A. The treatment of decorative tattoos with the carbon dioxide laser, 819
- LANIGAN, S. *see* DOWD, P.M.
- LARSEN, J.K. *see* RALFKAER, E.
- LASSUS, A. *see* REITAMO, S.
- LAWLOR, F., BARR, R.M., CROMELL, O., KOBZA BLACK, A. and GREAVES, M.W. Mediators in delayed pressure urticaria (Poster), 310
- LAWLOR, F., KOBZA BLACK, A., BREATHNACH, A.S. and GREAVES, M.W. Vibratory angioedema: lesion induction, clinical features, laboratory and ultrastructural findings and response to therapy, 93
- LAWLOR, F., KOBZA BLACK, A., MILFORD WARD, A., MORRIS, R. and GREAVES, M.W. Delayed pressure urticaria: objection evaluation of a variable disease using a dermatographometer and assessment of treatment using colchicine, 403
- LAWRENCE, C.M. *see* RAMSAY, B.
- LEAKE, R.E. *see* TILLMAN, D.M.
- LEE, T.H. *see* MARKEY, A.C.
- LEHTO, M. *see* RÄSÄNEN, L.
- LEIGH, I.M. *see* KERR, L.-A.P.
- LEIGH, I.M. *see* LEUNG, R.
- LEIGH, I.M. *see* NAVSARIA, H.
- LEIGH, I.M. *see* WRIGHT, S.
- LEMMING, R.J. *see* HAMON, C.G.B.
- LEUNG, R., RUSSELL JONES, R., SPAULL, J., LEIGH, I.M. and PURKIS, P. An immunohistochemical study of the effects of oral etretinate on the pattern of keratin expression of psoriatic skin using a panel of monoclonal antibodies specific for keratin subtypes (Poster), 305
- LEVELL, M.J., CAWOOD, M.L., BURKE, B. and CUNLIFFE, W.J. Acne is not associated with abnormal plasma antigens, 649
- LEVER, L. and MARKS, R. The significance of the Darier-like solar keratosis and acantholytic change in pre-neoplastic lesions of the epidermis, 363
- LEWIS, G. *see* ASHWORTH, J.
- LING, C.-S. *see* CHERN, L.-C.
- LIPSCOMB, G. *see* ZUMLA, A.
- LÖFBERG, H. *see* DAHLBÄCK, K.
- MACDONALD, D.M. *see* ALLEN, M.H.
- MACDONALD, D.M. *see* BARKER, J.N.W.N.
- MACDONALD, D.M. *see* HIGGINS, E.J.
- MACDONALD, D.M. *see* KERR L.-A.P.
- MACDONALD, D.M. *see* MARKEY, A.C.
- MACDONALD, D.M. *see* NAVSARIA, H.
- MACDONALD, D.M. *see* TIDMAN, M.J.
- MACDONALD, J.N. *see* SHIMIZU, H.
- MCDONALD, K. *see* GEBRIL, M.G.
- MACDONALD-HULL, S.P., COTTERILL, J.A.C. and NORRIS, J.F.B. Vitiligo following diphenylprone dermatitis (Correspondence), 323
- MACDONALD-HULL, S., GOODFIELD, M. and CUNLIFFE, W.J. The 'active' edge of psoriatic plaques: investigation by Laser-Doppler flowmetry and immunohistology (Poster), 306
- MCDOWELL, D. *see* GEBRIL, M.G.
- McFADDEN, J.P. *see* BAKER, B.S.
- McFADDEN, J. *see* GRIFFITHS, C.E.M.
- McGIBBOL, D.M. *see* NORRIS, P.G.
- McKERRROW, K.J., HAWTHORN, R.J. and THOMPSON, W. An investigation of circulating and *in situ* lymphocyte subsets in the skin and cervix of patients with chronic renal failure, 745
- MACKIE, R.M. *see* ASHWORTH, J.
- MACKIE, R.M. *see* TILLMAN, D.M.
- MACKIE, R.M. *see* EDWARD, M.
- MACLEOD, T.M. *see* MILLAR, B.
- MCLELLAND, J., MORRIS, J. and CHU, A.C. Comparison of the functional activities of Langerhans cells, blood dendritic cells and different monocyte populations using a handling droplet technique (Paper summary), 300
- MACNEIL, S. *see* HILL, S.E.
- MAIBACH, H. *see* BERARDESCA, E.
- MAJID, A., KNIGHT, R.A., COOKE, K.B. and COPEMAN, P.W.M. Anti-melanoma antibody analysis by enzyme-linked immunosorbent assay (ELISA) in diagnosis of benign and malignant pigmented skin lesions (Paper summary), 290
- MALI-GERRITS M. *see* VAN DE KERKHOFF, P.C.M.
- MALLET, R.B. *see* COOK, A.L.
- MARKEY, A.C. *see* CHURCHILL, L.J.
- MARKEY, A.C., BARKER, J.N.W.N., ARCHER, C.B., LEE, T.H. and MACDONALD, D.M. The clinical and histological effects of platelet activating factor (PAF-acether) in atopic skin, and the effect on response by the specific PAF-acether antagonist BN52063 (Paper summary), 287
- MARKEY, A.C., CHURCHILL, L.J. and MACDONALD, D.M. Human cutaneous mast cells—a study of fixative and staining reactions in normal skin, 625
- MARKEY, A.C. *see* ALLEN, M.H.
- MARKEY, A.C. *see* BARKER, J.N.W.N.
- MARKS, R., DYKES, P.J. and HAMAMI I. Two novel techniques for the evaluation of barrier creams, 665

- MARKS, R. *see* LEVER, L.
 MARKS, R. *see* MOURAD, M.M.
 MASSONE, L. *see* CROVATO, F.
 MATTHEWS, J.M. *see* TELFER, N.R.
 MATTHEWS, J.N.S. *see* REES, J.L.
 MAURICE, P.D. *see* ASHWORTH, J.
 MAYOU, S.C. *see* ASHERSON, R.A.
 MERRY, T.P. *see* ASHERSON, R.A.
 MIDGLEY, G. *see* RIVERS, J.K.
 MIER, P.D. Enzymatic diagnosis of congenital ichthyoses (Summary), 135
 MILES, C. *see* VAN HALEWYN, C.N.
 MILFORD WARD, A. *see* LAWLOR, F.
 MILLAR, B., GREEN, C., FERGUSON, J., RAFFLE, E.J. and MACLEOD, T.M. A study of the photodegradation of leukotriene B₄ by ultraviolet irradiation (UVB, UVA), 145
 MILLARD, P.R. *see* VENNING, V.A.
 MITCHELL, D.M. *see* MUSTAFA, Z.H.AL-
 MITCHELL, V.E. *see* BERTH-JONES, J.
 MIYAMOTO, T. *see* ASO, M.
 MOORE, M.K. and HAY, R.J. The specificity of antigen recognition in patients with dermatophyte infections (Paper summary), 299
 MORIMURA, T. *see* ASO, M.
 MORRIS, J., NORRIS, P. and CHU, A.C., UVB induced damage to the functional activity of epidermal Langerhans cells (Poster), 309
 MORRIS, J. *see* CHU, A.C.
 MORRIS, J. *see* McLELLAND, J.
 MORRIS, J. *see* NORRIS, P.G.
 MORRIS, J. *see* RIVERS, J.K.
 MORRIS, R. *see* LAWLOR, F.
 MORRIS, R.W. *see* RIVERS, J.K.
 MORTIMER, P.S. *see* COLVER, G.B.
 MOURAD, M.M., EDWARDS, C. and MARKS, R. The thickness and extensibility as indicators of disease severity in the gravitational syndrome (Poster), 311
 MUHLEMANN, M.F. *see* PORTER, C.D.
 MUKHTAR, H. *see* DON, P.S.C.
 MUNRO, C.S., DALY, H.M. and FRIEDMANN, P.S. Effects of terfenadine on response to house dust mite antigen in atopic eczema (Paper summary), 298
 MURPHY, G.M. *see* RIVERS, J.K.
 MUSTAFA, Z.H.AL-, WILLIAMS, F.M., HABOUBI, N.Y., MITCHELL, D.M., CHALMERS, R.J.G. and RAWLINS, M.D. Synthesis of methotrexate polyglutamates by human liver *in vivo* in patients receiving methotrexate for the treatment of severe psoriasis (Poster), 306
 NAKAJIMA, H. *see* ISHII, N.
 NASHIRO, K. *see* UEDA, Y.
 NAVSARIA, H., BARKER, J.N.W.N., MACDONALD, D.M. and LEIGH, I.M. Virally transformed human keratinocytes (SVK14 cells) as a model to study interferon-gamma induced keratinocyte class II MHC antigen biosynthesis (Paper summary), 303
 NAVSARIA, H.A. *see* KERR, I-A.P.
 NAZZARI, G. *see* CROVATO, F.
 NEILL, S.M. *see* COLVER, G.B.
 NEWTON, J.A., BOODLE, K.M., BARR, R., DOWD, P.M. and GREAVES, M.W. Topical nordihydroguaiaretic (NDGA) in psoriasis, 296
 NEWTON, J.A. *see* BUNKER, G.B.
 NEWTON, J.A. *see* RUSTIN, M.H.A.
 NICKOLOFF, B.J. *see* GRIFFITHS, C.E.M.
 NICOL, L. *see* TILLMAN, D.M.
 NOON, A.P. *see* GRATTAN, C.E.H.
 NORRIS, J.F.B. *see* MACDONALD-HULL, S.P.
 NORRIS, P.G., MORRIS, J., MCGIBBON, D.M., CHU, A.C. and HAWK, J.L.M. Polymorphic light eruption: an immunopathological study of evolving lesions, 173
 NORRIS, P.G. *see* RIVERS, T.K.
 O'DRISCOLL, J. *see* AUGUST, P.J.
 OPAS, B.E., ARGENTBRIGHT, L.W. and HUMES, J.L. An enzymatic method for distinguishing the stereoisomers of 12-hydroxyeicosatetraenoic acid in human epidermis and psoriatic scale, 49
 ORTONNE, J.P., TORZUOLI, C., DUJARDIN, P. and FRATAG, B. Ketanserin in the treatment of systemic sclerosis: a double-blind controlled trial, 261
 ORTONNE, J-P. *see* SCHOFIELD, O.M.V.
 OTSUKA, F. *see* UEDA, Y.
 PANAYI, G.S. *see* SAKKAS, L.I.
 PAPAMIMITRIOU, J.M. *see* GHANAWIE, M.
 PARKER, S.C. and HAMBLIN, A. Antigen-dependent epidermal cell-induced proliferation of human T cells is dependent on the purity of the T cells and the antigens used (Paper summary), 301
 PASINI, F. *see* BARBA, A.
 PATEL, A. *see* BUNKER, C.B.
 PAUL, C.J. *see* CARMICHAEL, A.J.
 PAYNE, P.A. and AYATOLLAHI, A. A new instrument for the measurement of skin blood flow using ultrasound Doppler techniques (Paper summary), 292
 PERRRET, C. Schöpf syndrome (Case report), 133
 PERRRET, C. Topical immunotherapy; indications and results (Summary), 137
 PHILLIPS, T. *see* WRIGHT, S.
 PHILPOT, C.M. *see* SHUTTLEWORTH, D.
 PICCIRILLI, A. *see* COSTAGLIOLA, C.
 PLEINIKKI, P. *see* RÄSÄNEN, L.
 POHLE, H.-D. *see* LANGFORD, A.
 PORTER, C.D., BLAKE, N.W., ARCHARD, L.C., MUHLEMANN, M.F., ROSEDALE, N. and CREAM, J.J. Molluscum contagiosum virus types in genital and non-genital lesions, 37
 POWLES, A.V. *see* BAKER, B.S.
 POWLES, A.V. *see* GRIFFITHS, C.E.M.
 PROTA, G. *see* RUOCO, V.
 PURKIS, P. *see* LEUNG, R.
 PUSKA, P. *see* REITAMO, S.
 PYE, R.J. *see* COOK, A.L.
 RAFFLE, E.J. *see* MILLAR, B.
 RALFKIAER, E., LARSEN, J.K., CHRISTENSEN, I.B.J., THOMSEN, K. and LANGE WANTZIN, G. DNA analysis by flow cytometry in cutaneous T-cell lymphomas, 597
 RAMSAY, B. and LAWRENCE, C.M. Comparison of subjective and objective methods of assessment of psoriasis plaque area (Paper summary), 293
 RANTALA, I. *see* RÄSÄNEN, L.
 RANZI, T., CAMPANINI, M. and BIANCHI, P.A. Successful treatment of genital and oral ulceration in Behcet's

- disease with topical 5-aminosalicylic acid (Correspondence), 471
- RÄSÄNEN, L., REUNALA, T., LEHTO, M., JANSEN, C., RANTALA, I. and PLEINIKKI, P. Immediate decrease in antigen-presenting function and delayed enhancement of interleukin-1 production in human epidermal cells after *in vivo* UVB irradiation, 589
- RAWLINS, D.M. *see* MUSTAFA, Z.H.A.L.
- READE, P.C. *see* RICH, A.M.
- RECHARDT, L. *see* VAALASTI, A.
- REES, J.L., FRIEDMANN, P.S. and MATTHEWS, J.N.S. Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene, 371
- REES, J. and FRIEDMANN, P.S. Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB) (Paper summary), 279
- REICHART, *see* LANGFORD, A.
- REITAMO, S., PUSKA, P. and LASSUS, A. Cyclosporin in the treatment of palmo-plantar pustulosis (Correspondence), 857
- REUNALA T. *see* RÄSÄNEN, L.
- RICH, A.M. and READE, P.C. A quantitative assessment of Langerhans cells in oral mucosal lichen planus and leukoplakia, 223
- RICHARDSON, M.D. *see* ALJABRE, S.J.
- RICHARDSON, S.G.N., CLARKE, C.W.F. and GASSON, G.B. Lymphocytic lymphoma and acquired C₁ esterase inhibitor deficiency, 121
- RIJZEWIJK, J. Congenital hereditary hypotrichosis of the Marie Unna type (Case report), 129
- RIVERS, J.K., NORRIS, P.G., MURPHY, G.M., CHU, A.C., MIDGLEY, G., MORRIS, J., MORRIS, R.W., YOUNG, A.R. and HAWK, J.L.M. UVA sunbeds: tanning, photoprotection, acute adverse effects and immunological changes, 767
- ROBINSON, T.W.E. *see* RUSTIN, M.H.A.
- RODGER, A. *see* COLVER, G.B.
- ROSDALE, N. *see* PORTER, C.D.
- ROSS, C.R. *see* CAMP, R.D.R.
- ROSS, J.S., BACON, K.B., and CAMP, R.D.R. Selective inhibition of lymphocyte chemotaxis by cyclosporin (Paper summary), 297
- ROUWELER, B.K. and JUIPER, J.P. Proteus syndrome (Case report), 129
- ROWLAND-PAYNE, C.M.E., CONNOLLY, C.N. and STAUGHTON, R.C.D. Intradermal lignocaine impairs subsequent *in vitro* growth of dermal fibroblasts (Poster), 308
- RUBINSTEIN, A., GOOR, Y., GAZIT, E. and CABILI S. Non-symmetric subcutaneous lipomatosis associated with familial combined hyperlipidaemia, 689
- RÜDLINGER, R., BUNNEY, M.H., GROB, R. and HUNTER, J.A.A. Warts in fish handlers, 375
- RUF, B. *see* LANGFORD, A.
- RUITER, D.J. Immunohistochemical analysis of melanocytic lesions (Summary), 137
- RUITER, D.J. *see* STEIJLEN, P.
- RUITER, D. *see* VAN DE KERKHOFF, P.C.M.
- RUOCCO, V., FLORIO, M., GRIMALDI FILIOLI, F., GUERRERA, V. and PROTA, G. An unusual case of trimethylaminuria, 459
- RUSSELL JONES, R. *see* ATHERTON, D.J.
- RUSSELL JONES, R. *see* LEUNG, R.
- RUSSELL JONES, R. *see* WHITTAKER, S.J.
- RUSTIN, M.H.A., BAINS, R. and DOWD, P.M. Epidermal class II antigen expression and Langerhans cell density in systemic sclerosis (Poster), 312
- RUSTIN, M.H.A., BULL, H.A. and DOWD, P.M. Effect of human recombinant interleukin-12 on the release of prostacyclin from human endothelial cells, 153
- RUSTIN, M.H.A., BUNKER, C.R. and DOWD, P.M. Chronic leg ulceration with livedoid vasculitis, and response to ketanserin, 101
- RUSTIN, M.H.A., BUNKER, C.B., DOWD, P.M. and ROBINSON, T.W.E. Erythema multiforme due to griseofulvin, 455
- RUSTIN, M.H.A., NEWTON, J.A., SMITH, N.P. and DOWD, P.M. The treatment of chilblains with nifedipine; the results of a pilot study, a double-blind placebo-controlled randomized study and a long-term open trial, 267
- RUSTIN, M.H.A. *see* BULL, H.A.
- RUSTIN, M.H.A. *see* DOWD, P.M.
- RYAN, J. *see* WRIGHT, S.
- RYAN, T.J. *see* BURGE, S.M.
- SAKKAS, L.I., DEMAINE, A., PANAYI, G.S. and WELSH, K.I. DNA variants at or near the IgH loci are associated with arthritis (PSA) in patients with psoriasis (Paper summary), 288
- SANDBANK, M. *see* DAVID, M.
- SARKANY, I. *see* DODD, H.J.
- SATO, K. *see* TAKEMURA, T.
- SAURAT, J-H. *see* HIRSCHSEL-SCHOLZ, S.
- SAVAGE, C.R. *see* BAKER, B.S.
- SAVIDGE, G.F. *see* HIGGINS, E.J.
- SAVIN, J.A. *see* COLVER, G.B.
- SCHENA, D. *see* BARBA, A.
- SCHOFIELD, O.M.V., HSI, B.L., YEH, C-J., FRED-REY-GROBELLET, D., ORTONNE, J-P. and EADY, R.A.J. GB1-monoclonal antibody indicates common antigen expression among human periderm and other tissues (Posters), 305
- SCHULER, G. *see* SEPP, N.
- SCHUURMAN STEKHOVEN, J. *see* VAN DE KERKHOFF, P.C.M.
- SCOTT, E.M. *see* ALJABRE, S.J.
- SCURO, L.A. *see* BARBA, A.
- SEKI, Y. *see* UEDA, Y.
- SEPP, N., HINNTER, H., SCHULER, G. and WICK, G. Dust- and rod-like particles in cutaneous biopsies of patients with systemic lupus erythematosus (Correspondence), 861
- SEVILLE, R.H. *see* WALKER, G.B.
- SHEHADE, S. *see* GEBRIL, M.G.
- SHIMAO, S. *see* ASO, M.
- SHIMIZU, H., MACDONALD, J.N., KENNEDY, A.R. and EADY, R.A.J. Comparison of two low-temperature techniques for the ultra-structural localization of epidermal basement membrane zone antigens (Poster), 304
- SHUSTER, S. *see* COWLEY, N.C.
- SHUTTLEWORTH, D., PHILPOT, C.M. and KNIGHT, A.G. Cutaneous cryptococcosis: treatment with oral fluconazole, 683
- SIEGENTHALER, G. *see* HIRSCHSEL-SCHOLZ, S.
- SKERROW, C.J. *see* KINN, S.R.

- SKERROW, C.J. *see* WILSON, W.D.
 SKERROW, D. *see* HUNTER, I.
 SKERROW, D. *see* KINN, S.R.
 SKERROW, D. *see* WILSON, W.D.
 SMITH, J.F. *see* WALKER, G.B.
 SMITH, N.P. *see* ATHERTON, D.J.
 SMITH, N. *see* CHU, A.C.
 SMITH, N.P. *see* RUSTIN, M.H.A.
 SMITH, N.P. *see* WILSON JONES, B.
 SMITH, N.P. *see* WHITTAKER, S.J.
 SNOWDEN, J.M. *see* CARTWRIGHT, P.H.
 SOYUER, U. *see* DOĞAN, P.
 SPAULL, J. *see* CERIO, R.
 SPAULL, J. *see* LEUNG, R.
 STAMP, G. *see* HALE, S.A.
 STANKLER, L. The use of water as a mounting medium for light microscopy to study the surface architecture of hair (Correspondence), 717
 STAUGHTON, R.C.D. *see* ROWLAND-PAYNE, C.M.E.
 STEIJLEN, P., HAMM, H., VAN ERP P., JOHNSON, J.P., RUITER, D.J. and BRÖCKER, E.B. Immunohistochemical evidence for the malignant potential of congenital melanocytic naevi, 137
 SUOMALAINEN, H. *see* VAALASTI, A.
 SUTO, T. *see* ISHII, N.
 SYMONDS, J.A. *see* KEMMETT, D.
 TAKEMURA, T., WERTZ, P.W. and SATO, K. Free fatty acids and sterols in human eccrine sweat, 43
 TAMAKI, K. *see* UEDA, Y.
 TANRIKULU, C. *see* DOĞAN, P.
 TAPSON, J.S. *see* COX, N.H.
 TAPLIN, J.D. *see* TIDMAN, M.J.
 TANI, K. *see* ISHII, N.
 TELFER, N.R., CUTLER, T.P. and DAWBER, R.P.R. Dystrophic pili torti (Correspondence), 323
 TELFER, N.R., MATTHEWS, J.N. and WOJNAROWSKA, F. Skin disease in haemophiliacs with and without antibodies to the human immunodeficiency virus (HIV): further evidence of disease behaviour in different risk groups?, 795
 TELLNER, D.C. *see* GUPTA, A.K.
 THOMPSON, W. *see* MCKERROW, K.J.
 THOMSEN, K. *see* RALFKIAER, E.
 TIDMAN, M.J., TAPLIN, J.D. and MACDONALD, D.M. Abnormal expression of G71 antigen at the epidermal basement membrane in basal cell carcinoma (Poster), 304
 TIDMAN, M.J. *see* HIGGINS, B.J.
 TILLMAN, D.M., NICOL, L., LEAKE, R.E., HODGINS, M.B. and MACKIE, R.M. Oestrogen receptors in congenital naevi (Poster), 308
 TOONSTRA, J., VAN DER PUTTE, S.C.J., VAN WICHEN, D.F., VAN WEELDEN, H., HENQUET, C.J.M. and VAN VLOTEN, W.A. Actinic reticuloid: immunohistochemical analysis of the cutaneous infiltrate in 13 patients, 779
 TÖRÖK, L., GALUSKA, L., KÁSA, M. and KÁDÁR, L. Bone-scintigraphic examinations in patients treated with retinoids: a prospective study, 31
 TORZUOLI, C. *see* ORTONNE, J.P.
 TURNER, D.M. *see* HOLLAND, D.B.
 TUYF, E. *see* ASHWORTH, J.
 UEDA, Y., NASHIRO K., SEKI, Y., OTSUKA, F., TAMAKI, K. and ISHIBASHI, Y. Pemphigoid vegetans, 449
 VAALASTI, A., SUOMALAINEN, H. and RECHARDT, L. Calcitonin gene-related peptide immunoreactivity in prurigo nodularis: a comparative study with neurodermatitis circumscripta, 619
 VALDIMARSSON, H. *see* BAKER, B.S.
 VALDIMARSSON, H. *see* GRIFFITHS, C.E.M.
 VAN BOKHOVEN, M. *see* VAN DE KERKHOFF, P.C.M.
 VAN DE KERKHOFF, P.C.M. What is new in psoriasis research? (Summary), 133
 VAN DE KERKHOFF, P.C.M., VAN BOKHOVEN, M., ZULTAK, M. and CZARNETZKI, B.M. A double-blind study of topical 1 α , 25-dihydroxy-vitamin D₃ in psoriasis, 661
 VAN DE KERKHOFF, P.C.M., MALI-GERRITS, M., RUITER, D. and SCHUURMAN STEKHOVEN, J. Keratosis pal-moplaris transgrediens et progrediens Greither, 125
 VAN DER MERWE, C.A. *see* HARDWICK, N.
 VAN DER PUTTE, S.C.J. *see* TOONSTRA, J.
 VAN DIJK, T. *see* VAN DOOREN-GREEBE, R.J.
 VAN DOOREN-GREEBE, R.J. and VAN DIJK, T. Congenital cutaneous ossification (Case report), 132
 VAN ERP, P. *see* STEIJLEN, P.
 VAN EYK, C.L.A. and VAN VLOTEN, W.A. Skin lesions due to exposure to toxic gases (Summary), 136
 VAN GELDER, L.W. *see* HARDWICK, N.
 VAN HALEWYN, C.N., MILES, C., BUTLER, P.H., HOLDS-WORTH, J. and WALKER, E. Rare-earth doped eyewear provide for safer laser surgery (Correspondence), 470
 VAN VLOTEN, W.A. *see* TOONSTRA, J.
 VAN VLOTEN, W.A. *see* VAN EYK, C.L.A.
 VAN WEELDEN, H. *see* TOONSTRA, J.
 VAN WICHEN, D.F. *see* TOONSTRA, J.
 VENNING, V.A., MILLARD, P.R. and WOJNAROWSKA, F. Dapsone as first line therapy for bullous pemphigoid, 83
 VOORHEES, J.J. *see* GRIFFITHS, C.E.M.
 VOORHEES, J.J. *see* GUPTA, A.K.
 WALKER, G.B., HARRISON, P.V., SEVILLE, R.H. and SMITH, J.F. Short contact dithranol therapy: advantages of a stiff emulsifying ointment base, 277
 WALKER, E.M. *see* FERGUSON, J.
 WARREN, J. *see* BERTH-JONES J.
 WEEMAES, C. *see* FLEUREN, E.
 WEISSMAN-KATZENELSON, V. *see* DAVID, M.
 WELLS, R.S. *see* ATHERTON, D.J.
 WELSH, K.I. *see* KERR L.A.P.
 WELSH, K.I. *see* SAKRAS, L.I.
 WERTZ, P.W. *see* TAKEMURA, T.
 WETZKER, R. *see* WOLLINA, U.
 WHITTAKER, S.J., SMITH, N.P. and RUSSELL JONES, R. Solitary morphoea profunda, 431
 WICK, G. *see* SEPP, N.
 WICKENS, D.G. *see* ASHWORTH, J.
 WICKS, A.C.B. *see* BERTH-JONES, J.
 WILLIAMS, F.M. *see* MUSTAFA, Z.H.AL-
 WILSON, W.D., SKERROW, C.J. and SKERROW, D. Quantitative comparison of intermediate filament formation from denatured and undenatured α -keratins (Poster), 307

- WILSON JONES, E., CERIO, R. and SMITH, N.P. Epithelioid cell histiocytoma: a new entity, 185
- WINKELMANN, R.K. *see* ALEGRE, V.A.
- WINKELMANN, R.K. *see* GRATTAN, C.E.H.
- WINKOVE, C. *see* GEBRIL, M.G.
- WOJNAROWSKA, F. *see* VENNING, V.A.
- WOJNAROWSKA, F. *see* KELLY, S.E.
- WOJNAROWSKA, F. *see* TELFER, N.R.
- WOLLINA, U., KNOPF, B., KLINGER, R. and KRETZKER, R. Calmodulin and psoriasis (Correspondence), 856
- Wong, C-K. *see* CHERN, L-C.
- WOOD, E.J. *see* HOLLAND, D.B.
- WOODMAN, J.P. The host response to experimental infection with *Dermatophilus congolensis*—a skin pathogen of animals (Paper summary), 298
- WRIGHT, S., PHILLIPS, T., RYAN, J. and LEIGH, I.M. Intra-epidermal neutrophilic IgA dermatosis with colitis, 113
- WYATT, E.H. *see* ABDULLAH, A.N.
- YARDLEY, H.J. *see* CARTWRIGHT, H.J.
- YEH, C-J. *see* SCHOFIELD, O.M.V.
- YOUNG, A.R. *see* RIVERS, J.K.
- ZOLLMAN, P.E. *see* GRATTAN, C.E.H.
- ZULTAK, M. *see* VAN DE KERKHOF, P.C.M.
- ZÚMLA, A. and LIPSCOMB, G. Purpura and sepsis—rarer causes? (Correspondence), 715

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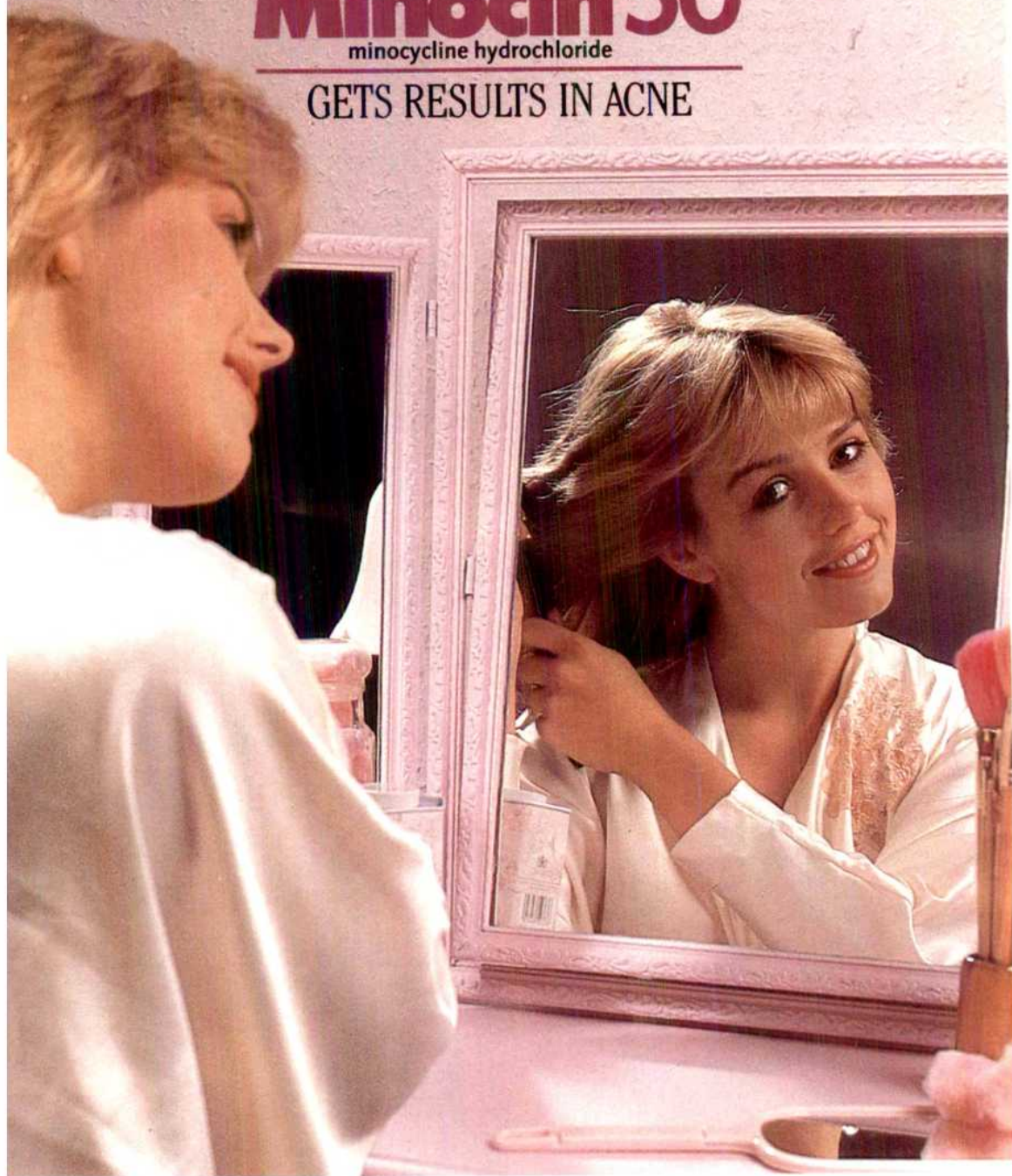
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Contents

No. 1: JANUARY 1989

Gamma-interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens	1
C.E.M. GRIFFITHS, J.J. VOORHEES AND B.J. NICKOLOFF	
Keratin gene expression during the resolution of psoriatic plaques: effects of dithranol, PUVA, etretinate and hydroxyurea regimens	9
D.B. HOLLAND, E.J. WOOD, W.J. CUNLIFFE AND D.M. TURNER	
1 α -25-dihydroxyvitamin D ₃ increases intracellular free calcium in murine B16 melanoma	21
S.E. HILL, S.S. BLEEHEN AND S. MACNEIL	
Bone-scintigraphic examinations in patients treated with retinoids: a prospective study	31
L. TÖRÖK, L. GALUSKA, M. KÁSA AND L. KÁDÁR	
Molluscum contagiosum virus types in genital and non-genital lesions	37
C.D. PORTER, N.W. BLAKE, L.C. ARCHARD, M.F. MUHLEMANN, N. ROSEDALE AND J.J. CREAM	
Free fatty acids and sterols in human eccrine sweat	43
T. TAKEMURA, P.W. WERTZ AND K. SATO	
An enzymatic method for distinguishing the stereoisomers of 12-hydroxyeicosatetraenoic acid in human epidermis and psoriatic scale	49
B.E. OPAS, L.W. ARGENTBRIGHT AND J.L. HUMES	
Binding of bullous pemphigoid and pemphigus vulgaris sera to SCaBER cell line in an ELISA	59
A.L. COOKE, R.B. MALLET, T.H.P. HANAHOE AND R.J. PYE	
Intestinal permeability in patients with atopic eczema	71
A. BARBA, D. SCHENA, M.C. ANDREAUS, G. FACCINI, F. PASINI, G. BROCCO, G. CAVALLINI, L.A. SCURO AND G.C. CHIERE-GATO	
An epidemiological comparison between occupational and non-occupational hand eczema	77
G.L. GOH	
Dapsone as first line therapy for bullous pemphigoid	83
V.A. VENNING, P.R. MILLARD AND F. WOJNAROWSKA	
Vibratory angioedema: lesion induction, clinical features, laboratory and ultrasound findings and response to therapy	93
FRANCES LAWLOR, ANNE KOBZA BLACK, A.S. BREATHNACH AND M.W. GREAVES	
Chronic leg ulceration with livedoid vasculitis, and response to oral ketanserin	101
M.H.A. RUSTIN, C.B. BUNKER AND PAULINE M. DOWD	
Acute acral pustulosis	107
T.J. HOFFMANN, ANNE KETTLER AND SUZANNE BRUCE	
Intra-epidermal neutrophilic IgA dermatosis with colitis	113
S. WRIGHT, T. PHILLIPS, J. RYAN AND I.M. LEIGH	
Lymphocytic lymphoma and acquired C ₁ esterase inhibitor deficiency	121
S.G.N. RICHARDSON, C.W.F. CLARKE AND G.B. GASSON	
Society Proceedings	
Proceedings of the 242nd Meeting of the Netherlands Society for Dermatology and Venereology	125
Correspondence	
Great toe-nail dystrophy	139
T.J.A. DAWSON AND R. BARAN	
Congenital epidermolytic hyperkeratosis associated with multiple malignancies	141
J.M. EDWARDS, M.A.C.S. COOPER AND S. BANNERJEE	

No. 2: FEBRUARY 1989

A study of the photodegradation of leukotriene B ₄ by ultraviolet irradiation (UVB, UVA)	145
B.MILLAR, C.GREEN, J.FERGUSON, E.J.RAFFLE AND T.M.MACLEOD	
Effect of human recombinant interleukin-1 α on release of prostacyclin from human endothelial cells	153
M.H.A.RUSTIN, HELEN.A.BULL AND PAULINE M.DOWD	
Benzo(a)pyrene metabolism, DNA-binding and UV-induced repair of DNA damage in cultured skin fibroblasts from a patient with unilateral multiple basal cell carcinoma	161
P.S.C.DON, H.MUKHTAR, M.DAS, N.A.BERGER AND D.R.BICKERS	
Polymorphic light eruption: an immunopathological study of evolving lesions	173
P.G.NORRIS, JENNY MORRIS, D.M.MCGIBBON, A.C.CHU AND J.L.M.HAWK	
Epithelioid cell histiocytoma: a new entity	185
E.WILSON JONES, R.CERIO AND N.P.SMITH	
Histiocytoma cutis: a tumour of dermal dendrocytes (dermal dendrocytoma)	197
R.CERIO, J.SPAULL AND E.WILSON JONES	
Intralesional cyclosporin in psoriasis: effects on T lymphocyte and dendritic cell subpopulations	207
BARBARA S.BAKER, A.V.POWLES, C.R.SAVAGE, J.P.MCFADDEN, H.VALDIMARSSON AND L.FRY	
The spectrum of livedo reticularis and anticardiolipin antibodies	215
R.A.ASHERSON, S.C.MAYOU, P.MERRY, M.M.BLACK AND G.R.V.HUGHES	
A quantitative assessment of Langerhans cells in oral mucosal lichen planus and leukoplakia	223
ALISON M.RICH AND P.C.READE	
Exogenous ochronosis: an epidemiological study	229
N.HARDWICK, L.W.VAN GELDER, C.A.VAN DER MERWE AND M.P.VAN DER MERWE	
Superoxide dismutase and myeloperoxidase activity in polymorphonuclear leukocytes, and serum ceruloplasmin and copper levels, in psoriasis	239
P.DOĞAN, Ü.SOYUER AND G.TANRIKULU	
Atopic dermatitis and house dust mites	245
H-I.BECK AND J.KORSGAARD	
Long-term cyclosporin for psoriasis	253
C.E.M.GRIFFITHS, A.V.POWLES, J.MCFADDEN, B.S.BAKER, H.VALDIMARSSON AND L.FRY	
Ketanserin in the treatment of systemic sclerosis: a double-blind controlled trial	261
J.P.ORTONNE, C.TORZUOLI, P.DUJARDIN AND B.FRAITAG	
The treatment of chilblains with nifedipine: the results of a pilot study, a double-blind placebo-controlled randomized study and a long-term open trial	267
M.H.A.RUSTIN, JULIA A.NEWTON, N.P.SMITH AND PAULINE M.DOWD	
Short contact dithranol therapy: advantages of a stiff emulsifying ointment base	277
G.B.WALKER, P.V.HARRISON, R.H.SEVILLE AND J.F.SMITH	
Society Proceedings	
British Society for Investigative Dermatology Annual Meeting, Manchester, September 1988	285
Obituary	
Dr Owen Finn	315
Correspondence	
Reversible gynaecomastia associated with etretinate	317
A.J.CARMICHAEL AND C.J.PAUL	
Meyerson's naevi in pityriasis rosea	318
F.CROVATO, G.NAZZARI, C.GAMBINI AND L.MASSONE	
Lichen planus associated with captopril: a further disorder demonstrating the 'tin-tack' sign	319
N.H.COX, J.S.TAPSON AND P.M.FARR	
Platelet hyperaggregability in two patients with Klinefelter's syndrome complicated by leg ulcers	322
E.J.HIGGINS, M.J.TIDMAN, G.F.SAVIDGE, J.BEARD AND D.M.MACDONALD	
Vitiligo following diphenylprone dermatitis	323
S.P.MACDONALD-HULL, J.A.C.COTTERILL AND J.F.B.NORRIS	
The natural history of 'dystrophic' pili torti	323
N.R.TELFER, T.P.CUTLER AND R.P.R.DAWBER	
Book Reviews	327
News and Notices	327

No. 3: MARCH 1989

PUVA therapy decreases HLA-DR ⁺ CD1a ⁺ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA-DR ⁺ CD1a ⁺ Langerhans cells exhibit normal alloantigen-presenting function	329
J.ASHWORTH, M.C.KAHAN AND S.M.BREATHNACH	
Reduced density and morphologic alteration of Ia and ADPase positive Langerhans cells after low-protein diet	341
M.GHAZNAWIE, J.M.PAPADIMITRIOU AND P.J.HEENAN	
Identification of the origin of cells in human basal cell carcinoma xenografts in mice using <i>in situ</i> hybridization	351
S.A.HALES, G.STAMP, M.EVANS AND K.A.FLEMING	
Infantile seborrheic dermatitis and <i>Pityrosporum ovale</i>	359
ANN BROBERG AND JAN FAERGEMANN	
Relationship of the final α -keratin compositions in dyskeratoses to those along the normal keratinization pathway: aetiological and diagnostic significance	363
I.HUNTER AND D.SKERROW	
Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB)	371
J.L.REES, P.S.FRIEDMANN AND J.N.S.MATTHEWS	
Warts in fish handlers	375
R.RÜDLINGER, MARY H.BUNNEY, R.GROB AND J.A.A.HUNTER	
The significance of the Darier-like solar keratosis and acantholytic change in preneoplastic lesions of the epidermis	383
L.LEVER AND R.MARKS	
The usefulness of immunofluorescent tests in pemphigus patients in clinical remission	391
M.DAVID, V.WEISSMAN-KATZENELSON, A.BEN-CHETRIT, B.HAZAZ, A.INGBER AND M.SANDBANK	
Allergic and irritant contact dermatitis compared in the treatment of alopecia totalis and universalis. A comparison of the value of topical diphenylprone and tretinoin gel	397
J.ASHWORTH, E.TUYP AND RONA M.MACKIE	
Delayed pressure urticaria, objective evaluation of a variable disease using a dermatographometer and assessment of treatment using colchicine	403
FRANCES LAWLOR, ANNE KOBZA BLACK, A.MILFORD WARD, R.MORRIS AND M.W.GREAVES	
Urticaria successfully treated by desensitization with grass pollen extract	409
P.J.AUGUST AND J.O'DRISCOLL	
Treatment of actinic prurigo with PUVA: mechanism of action	411
P.M.FARR AND B.L.DIFFEY	
Skin lesions associated with circulating lupus anticoagulant	419
V.A.ALEGRE, D.A.GASTINEAU AND R.K.WINKELMANN	
Solitary morphoea profunda	431
S.J.WHITTAKER, N.P.SMITH AND R.RUSSELL JONES	
Sneddon's syndrome (livedo reticularis and cerebral thrombosis) with livedo vasculitis and anticardiolipin antibodies	441
C.E.H.GRATTAN, J.L.BURTON AND A.P.BOON	
Pemphigoid vegetans	449
Y.UEDA, K.NASHIRO, Y.SEKI, F.OTSUKA, K.TAMAKI AND Y.ISHIBASHI	
Erythema multiforme due to griseofulvin	455
M.H.A.RUSTIN, C.B.BUNKER, PAULINE M.DOWD AND T.W.E.ROBINSON	
An unusual case of trimethylaminuria	459
V.RUCCO, M.FLORIO, F.GRIMALDI FILIOLI, V.GUERRERA AND G.PROTA	
A family with Dowling Degos disease showing features of Kitamura's reticulate acropigmentation	463
J.BERTH-JONES AND R.A.C.GRAHAM-BROWN	
Obituary	
Dr H.W.Gordon	467
Correspondence	
Discoid lupus, erythematosus associated with the anticardiolipin syndrome	469
J.BERTH-JONES, P.E.HUTCHINSON, A.C.B.WICKS, J.WARREN AND V.E.MITCHELL	
Rare-earth doped eyewear provide for safer laser surgery	470
C.N.VAN HALEWYN, CAROL MILES, P.H.BUTLER, J.HOLDSWORTH AND E.WALKER	
Successful treatment of genital and oral ulceration in Behcet's disease with topical 5-aminosalicylic acid (5-ASA)	471
T.RANZI, M.CAMPANINI AND P.A.BIANCHI	
Cataracts associated with long-term topical steroids	472
C.COSTAGLIOLA, B.CATTI-GIOVANNELLI, A.PICCIRILLO AND M.DELFINO	

Seventy years on: 3, St Andrew's Place S.GOLD	473
Book Reviews	475
News and Notices	478

No. 4: APRIL 1989

Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation S.LISBY, E.RALFKIAER, R.ROTHLEIN AND G.L.VEJLSGAARD	479
A flow cytometric study of Langerhans cell histiocytosis JANET MCLELLAND, JULIA A. NEWTON, MARIAN MALONE, R.S.CAMPLEJOHN AND A.C.CHU	485
Suppression of contact sensitivity by local hyperthermia treatment due to reduced Langerhans cell population in mice A.YOSHIOKA, Y.MIYACHI, S.IMAMURA, M.HIRAOKA, S.JO AND M.ABE	493
Triiodothyronine alters the cornification of cultured human keratinocytes R.R.ISSEROFF, K.T.CHUN AND R.M.ROSENBERG	503
An assessment of the value of AgNOR staining in the identification of dysplastic and other borderline melanocytic naevi RONA M.MACKIE, S.I.WHITE, MORAG M.SEYWRIGHT AND H.YOUNG	511
The normal range in diagnostic phototesting B.L.DIFFEY AND P.M.FARR	517
Inhibition of proliferation of HeLa cells by dithranol (anthralin) and 10-butyl dithranol (butantrone)... ANITA REMITZ, ARJA-LEENA KARINEMI, EERO LEHTONEN AND STIG NORDLING	525
Identification of the epidermolysis bullosa acquisita antigen by LH 7-2 monoclonal antibody: use in diagnosis F.M.TATNALL, P.C.WHITEHEAD, M.M.BLACK, F.WOJNAROWSKA AND I.M.LEIGH	533
The pathogenesis of lichen planus A.GILHAR, T.PILLAR, G.WINTERSTEIN AND A.ETZIONI	541
Pityriasis rotunda as a cutaneous marker of hepatocellular carcinoma: a comparison with its prevalence in other diseases I.BERKOWITZ, H.J.HODKINSON, M.C.KEW AND A.M.DIBISCEGLIE	545
Epidermal dysplasia and cyclosporine therapy in renal transplant patients: a comparison with azathioprine D.SHUTTLEWORTH, R.MARKS, P.J.A.GRIFFIN AND J.R.SALAMAN	551
Sunbed-induced pseudoporphyria G.M.MURPHY, J.WRIGHT, D.S.H.NICHOLLS, P.H.MCKEE, A.G.MESSENGER, J.L.M.HAWK AND G.M.LEVENE	555
Brawny cutaneous induration and granulomatous panniculitis in large cell non-Hodgkin's (T suppressor/cytotoxic cell) lymphoma J.ASHWORTH, A.T.COADY, R.GUY AND S.M.BREATHNACH	563
Nail dystrophy in cutaneous T-cell lymphoma K.DALZIEL, N.R.TELFER AND R.P.R.DAWBER	571
Kaposi-like acroangiodermatitis in an above-knee amputation stump G.KOLDE, J.WÖRHEIDE, R.BAUMGARTNER AND EVA-B. BRÖCKER	575
Correspondence	
Topical eicosapentaenoic acid (EPA) in the treatment of psoriasis CATHERINE E.DEWSBURY, PAMELA GRAHAM AND C.R.DARLEY	581
Drug interactions with methotrexate B.J.LIDDLE AND J.R.MARSDEN	582
Fixed drug eruptions: 77 cases from 1981 to 1985 SAKARI STUBB, KRISTINA ALANKO AND SAKARI REITAMO	583
Psoriasis: improvement during and worsening after pregnancy S.F.DUNNA AND A.Y.FINLAY	584
Book Reviews	585
News and Notices	587

Immediate decrease in antigen-presenting function and delayed enhancement of interleukin-1 production in human epidermal cells after <i>in vivo</i> UVB irradiation	589
L.RÄSÄNEN, T.REUNALA, M.LEHTO, C.JANSÉN, I.RANTALA AND P.LEINIKKI	
DNA analysis by flow cytometry in cutaneous T-cell lymphomas	597
ELISABETH RALFKIAER, JØRGEN K.LARSEN, IB J.CHRISTENSEN, KRISTIAN THOMSEN AND GUNHILD LANGE WANTZIN	
Peripheral blood mononuclear leukocyte cyclic adenosine monophosphate specific phosphodiesterase activity in childhood atopic dermatitis	607
I.H.COULSON, S.N.DUNCAN AND C.A.HOLDEN	
Keratinocyte expression of OKM5 antigen in inflammatory cutaneous disease	613
J.N.W.N.BARKER, A.C.MARKEY, M.H.ALLEN AND D.M.MACDONALD	
Calcitonin gene-related peptide immunoreactivity in prurigo nodularis: a comparative study with neurodermatitis circumscripta	619
ANNIKKI VAALASTI, HANNA SUOMALAINEN AND LEENA RECHARDT	
Human cutaneous mast cells—a study of fixative and staining reactions in normal skin	625
A.C.MARKEY, LINDA J.CHURCHILL AND D.M.MACDONALD	
Multiple infundibular tumours of the head and neck	633
G.H.FINDLAY	
Isotretinoin differs from other synthetic retinoids in its modulation of human cellular retinoic acid binding protein (CRABP)	639
SUSANNE HIRSCHEL-SCHOLZ, G.SIEGENTHALER AND JEAN-HILAIRE SAURAT	
An investigation into the incidence of auto-immune disorders in patients with localized morphoea	645
CHRISTINE I.HARRINGTON AND I.R.DUNSMORE	
Acne is not associated with abnormal plasma androgens	649
M.J.LEVELL, M.L.CAWOOD, B.BURKE AND W.J.CUNLIFFE	
Two novel techniques for the evaluation of barrier creams	655
R.MARKS, P.J.DYKES AND I.HAMAMI	
A double-blind study of topical 1 α , 25-dihydroxyvitamin D ₃ in psoriasis	661
P.C.M.VAN DE KERKHOF, M.VAN BOKHOVEN, M.ZULTAK AND B.M.CZARNETZKI	
Combined treatment of psoriasis with acitretin and UVB phototherapy compared with acitretin alone and UVB alone	665
J.IEST AND J.BOER	
Familial keratoses of actinic distribution associated with internal malignancy and cellular hypersensitivity to UVA	671
D.J.ATHERTON, P.K.BOTCHERBY, A.J.FRANCIS, N.P.SMITH, R.S.WELLS AND F.GIANNELLI	
Cutaneous cryptococcosis: treatment with oral fluconazole	683
D.SHUTTLEWORTH, C.M.PHILPOT AND A.G.KNIGHT	
Non-symmetric subcutaneous lipomatosis associated with familial combined hyperlipidaemia	689
A.RUBINSTEIN, Y.GOOR, E.GAZIT AND S.CABILI	
Cutaneous chylous reflux	695
L-C.CHERN, C-S.LIN AND C-K.WONG	
Imported rickettsial African spotted fever in Japan	701
N.ISHII, H.KOMATSU, H.NAKAJIMA, K.TANI AND T.SUTO	
Prurigo pigmentosa successfully treated with minocycline	705
M.ASO, T.MIYAMOTO, T.MORIMURA AND S.SHIMAO	
Regression of oral Kaposi's sarcoma in a case of AIDS on Zidovudine (AZT)	709
A.LANGFORD, B.RUF, R.KUNZE, H-D.POHLE AND P.REICHART	
Correspondence	
Premenstrual exacerbation of atopic dermatitis	715
D.KEMMETT	
Purpura and sepsis—rarer causes?	715
A.ZUMLA AND G.LIPSCOMB	
Concomitant Becker's naevus and acanthosis nigricans	716
R-F.H.J.HULSMANS AND F-J.H.HULSMANS	
The use of water as a mounting medium for light microscopy to study the surface architecture of hair	717
L.STANKLER	
Book Reviews	719
News and Notices	722
Erratum	723

Vitronectin colocalizes with Ig deposits and C9 neoantigen in discoid lupus erythematosus and dermatitis herpetiformis, but not in bullous pemphigoid	725
KARIN DAHLBÄCK, HELGE LÖFBERG AND BJÖRN DAHLBÄCK	
Immunopathology of the placenta in pemphigoid gestationis and linear IgA disease	735
SUSAN E.KELLY, S.FLEMING, B.S.BHOGAL, F.WOJNAROWSKA AND M.M.BLACK	
An investigation of circulating and <i>in situ</i> lymphocyte subsets and Langerhans cells in the skin and cervix of patients with chronic renal failure	745
K.J.MCKERROW, R.J.HAWTHORN AND W.THOMPSON	
Release of prostacyclin from cultured human endothelial cells following prolonged exposure to histamine	757
HELEN A.BULL, M.H.A.RUSTIN AND PAULINE M.DOWD	
UVA sunbeds: tanning, photoprotection, acute adverse effects and immunological changes	767
J.K.RIVERS, P.G.NORRIS, G.M.MURPHY, A.C.CHU, G.MIDGLEY, J.MORRIS, R.W.MORRIS, A.R.YOUNG AND J.L.M.HAWK	
Actinic reticuloid: immunohistochemical analysis of the cutaneous infiltrate in 13 patients	779
J.TOONSTRA S.C.J.VAN DER PUTTE, D.F.VAN WICHEN, H.VAN WEELDEN, C.J.M.HENQUET AND W.A.VAN VLOTEN	
Cutaneous reactive hyperaemia: racial differences induced by corticoid application	787
E.BERARDESCA AND H.MAIBACH	
Skin diseases in haemophiliacs with and without antibodies to the human immunodeficiency virus (HIV): further evidence of altered disease behaviour in different risk groups?	795
N.R.TELFER, J.M.MATTHEWS AND F.WOJNAROWSKA	
Double-blind, placebo-controlled study to evaluate the efficacy of fish oil and low-dose UVB in the treatment of psoriasis	801
A.K.GUPTA, C.N.ELLIS, D.C.TELLNER, T.F.ANDERSON AND J.J.VOORHEES	
The short-term benefit and long-term failure of ultraviolet light in the treatment of venous leg ulcers	809
H.J.DODD, I.SARKANY AND P.M.GAYLARDE	
The treatment of decorative tattoos with the carbon dioxide laser	819
S.W.LANIGAN, R.A.SHEEHAN-DARE AND J.A.COTTERILL	
Darier's disease with peri-follicular depigmentation	827
J.BERTH-JONES AND P.E.HUTCHINSON	
Post-irradiation morphoea	831
G.B.COLVER, A.RODGER, P.S.MORTIMER, J.A.SAVIN, S.M.NEILL AND J.A.A.HUNTER	
Naevoid psoriasis	837
D.J.ATHERTON, M.KAHANA AND R.RUSSELL-JONES	
Skin necrosis in giant cell (temporal) arteritis: report of three cases	847
A.N.ABDULLAH, K.KECZKES AND E.H.WYATT	
Obituary	
Dr Louis Forman	847
Correspondence	
Dust- and rod-like particles in cutaneous biopsies of patients with systemic lupus	851
N.SEPP, H.HINTNER, G.SCHULER AND G.WICK	
Attempted passive transfer of a serum factor in chronic urticaria from human to monkey	853
C.E.H.GRATTAN, R.K.WINKELMANN AND P.E.ZOLLMAN	
Calmodulin and psoriasis	856
U.WOLLINA, B.KNOPF, R.KLINGER AND R.WETZKER	
Cyclosporin in the treatment of palmo-plantar pustulosis	857
S.REITAMO, P.PUSKA AND A.LASSUS	
Fixed drug reactions to pseudoephedrine hydrochloride	858
C.CAMISA	
Book Reviews	859
News and Notices	861
Index	863

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	<i>g</i>
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometre(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hours(s)	h	milliequivalent	mmol		

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CONTENTS

PAGE

Vitronectin colocalizes with Ig deposits and C9 neoantigen in discoid lupus erythematosus and dermatitis herpetiformis, but not in bullous pemphigoid	725
KARIN DAHLBÄCK, HELGE LÖFBERG AND BJÖRN DAHLBÄCK	
Immunopathology of the placenta in pemphigoid gestationis and linear IgA disease	735
SUSAN E. KELLY, S. FLEMING, B. S. BHOGAL, F. WOJNAROWSKA AND M. M. BLACK	
An investigation of circulating and <i>in situ</i> lymphocyte subsets and Langerhans cells in the skin and cervix of patients with chronic renal failure	745
K. J. MCKERROW, R. J. HAWTHORN AND W. THOMPSON	
Release of prostacyclin from cultured human endothelial cells following prolonged exposure to histamine	757
HELEN A. BULL, M. H. A. RUSTIN AND PAULINE M. DOWD	
UVA sunbeds: tanning, photoprotection, acute adverse effects and immunological changes	767
J. K. RIVERS, P. G. NORRIS, G. M. MURPHY, A. C. CHU, G. MIDGLEY, J. MORRIS, R. W. MORRIS, A. R. YOUNG AND J. L. M. HAWK	
Actinic reticuloid: immunohistochemical analysis of the cutaneous infiltrate in 13 patients	779
J. TOONSTRA S. C. J. VAN DER PUTTE, D. F. VAN WICHEN, H. VAN WEELDEN, C. J. M. HENQUET AND W. A. VAN VLOTEN	
Cutaneous reactive hyperaemia: racial differences induced by corticoid application	787
E. BERARDESCA AND H. MAIBACH	
Skin diseases in haemophiliacs with and without antibodies to the human immunodeficiency virus (HIV): further evidence of altered disease behaviour in different risk groups?	795
N. R. TELFER, J. M. MATTHEWS AND F. WOJNAROWSKA	
Double-blind, placebo-controlled study to evaluate the efficacy of fish oil and low-dose UVB in the treatment of psoriasis	801
A. K. GUPTA, C. N. ELLIS, D. C. TELLNER, T. F. ANDERSON AND J. J. VOORHEES	
The short-term benefit and long-term failure of ultraviolet light in the treatment of venous leg ulcers	809
H. J. DODD, I. SARKANY AND P. M. GAYLARDE	
The treatment of decorative tattoos with the carbon dioxide laser	819
S. W. LANIGAN, R. A. SHEEHAN-DARE AND J. A. COTTERILL	
Darier's disease with peri-follicular depigmentation	827
J. BERTH-JONES AND P. E. HUTCHINSON	
Post-irradiation morphoea	831
G. B. COEYER, A. RODGER, P. S. MORTIMER, J. A. SAVIN, S. M. NEILL AND J. A. A. HUNTER	
Naevoid psoriasis	837
D. J. ATHERTON, M. KAHANA AND R. RUSSELL-JONES	
Skin necrosis in giant cell (temporal) arteritis: report of three cases	843
A. N. ABDULLAH, K. KECZKES AND E. H. WYATT	
Obituary	
Dr Louis Forman	847
Correspondence	
Dust- and rod-like particles in cutaneous biopsies of patients with systemic lupus	851
N. SEPP, H. HINTNER, G. SCHULER AND G. WICK	
Attempted passive transfer of a serum factor in chronic urticaria from human to monkey	853
C. E. H. GRATTAN, R. K. WINKELMANN AND P. E. ZOLLMAN	
Calmodulin and psoriasis	856
Ü. WOLLINA, B. KNOPE, R. KLINGER AND R. WETZKER	
Cyclosporin in the treatment of palmo-plantar pustulosis	857
S. REITAMO, P. PUSKA AND A. LASSUS	
Fixed drug reactions to pseudoephedrine hydrochloride	858
C. CAMISA	
Book Reviews	859
News and Notices	861
Index	863

